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## EARLY VIGOR OF MAIZE PLANTS AND YIELD OF GRAIN AS INFLUENCED BY THE CORN ROOT, STALK, AND EAR ROT DISEASES<sup>1</sup>

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### PURPOSE OF THE PAPER

The purpose of this paper is (1) to emphasize the important relation existing between early vigor and yield of dent (*Zea mays indentata*), (2) to call attention to the reduction in early vigor of corn plants grown from seed infected with one or more of the corn root and stalk rot pathogenes or seed susceptible to the root and stalk rots, and (3) to present data bearing directly on the correlation between this reduced early vigor and reduced yield, with special reference to reduced yield of marketable corn.

A study of any field of corn, especially during the first few weeks after the plants have appeared above the ground, reveals striking differences in plant vigor. These differences in early vigor may be caused by many factors and may express themselves in both the size and appearance of the plants.

### REVIEW OF LITERATURE

Darwin (7)<sup>3</sup> found that maize plants grown from self-fertilized seed were slower growing and less vigorous than plants grown from cross-fertilized seed. Beal, of the Michigan Agricultural Experiment Station (28), in 1877-1882 reported an increase in both vigor and yield in first-generation hybrids when certain unrelated varieties were crossed. He urged the commercial utilization of this increased vigor as a method of increasing yields. The results of the early experiments of Beal were confirmed by other workers. Collins (5, 6) found that many crosses between diverse types of maize resulted in greatly increased vigor, a wider range of adaptability, and increased productivity.

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<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," p. 628-639.

Studies by East (9, 10), Shull (36), East and Hayes (11), Jones (24), and East and Jones (12), with inbred strains or biotypes, showed that one of the effects of inbreeding in maize was a reduction of vigor and yield. Crossing unrelated inbred strains greatly increased vigor and yield. They (11) state that "inbreeding always reduced the yield of seed and the height and delayed the time of flowering." These workers pointed out that reduction in vigor due to inbreeding may or may not be accompanied by pathologic symptoms (11). An illuminating discussion on inbreeding and hybrid vigor, or heterosis, together with an extensive bibliography, is given by East and Jones (12).

In his investigations of the chlorophyll inheritance in maize, Lindstrom (27) observed "a general correlation between chlorophyll factors and plant growth."

Ewing (13) obtained the following correlations with Funk Yellow Dent:

Weight of grain and diameter of stalk.....	+0.393	±0.020
Weight of grain and breadth of leaf.....	+0.314	±0.021
Weight of grain and height of seedlings.....	+0.219	±0.037
Weight of grain and height of mature plants.....	+0.203	±0.025

He expressed the belief that very little use could be made of correlation in practical corn breeding. He stated that there were rare cases in which the coupling of unit characters may assist the breeder in making selections at an early period of growth, but that the correlation in the fluctuating variability of two different characters is not likely to prove of much assistance.

Pearl and Surface (30) made a study of growth and variability in sweet corn in the summer of 1908. Growth measurements of a large number of plants were made at intervals of three and one-half days, beginning on June 11 and continuing until growth was completed. They found that the relative variability of height decreased as the season advanced and was on the average about 10 per cent lower in the matured plants than at the beginning of the season. During the tasseling period a marked increase in variability was noted, followed by a gradual decline. Their experiments demonstrated that plants relatively vigorous or weak at the beginning of the season tend to retain this comparative vigor. They regarded the differences in manner of growth as due to independent Mendelian factors distributed at random in the population of open-fertilized plants.

Reed (31) studied the growth of a number of sunflower plants (*Helianthus annuus* L.). Measurements of the plants were made in centimeters at 7-day intervals during the greatest period of growth. The plants were grouped in four equal numerical divisions called quartiles, on the basis of size. The season's study of the plants in these quartiles showed a well marked tendency to remain in a given quartile during the entire period of growth. Plants small at maturity generally had been small from the beginning and those large at maturity had a well-marked superiority from the start. Reed (31) found, as did Surface and Pearl (30), that height was determined by independent height factors distributed at random through the segregating population which he studied.

Hughes (22) made extensive measurements of plants during two years, involving 828 ear rows. He found that plants highest at the first measurement made the most rapid average height increment per plant and produced the greatest yield. Plants from horny kernels made a more rapid height increment than plants from starchy kernels and also produced

the greater yields. Kyle (26) found that corn plants making a vigorous early growth gave greater yields than those that were weak in their early stages of development. Grantham (14) studied the development and maturity of 100 hills of corn where a weak plant was growing with a vigorous one. From 50 of the hills he removed the weak plant and from 50 others he removed the vigorous plant. Measurements taken at different dates showed that the vigorous plants made the most rapid early growth and the greatest yield of shelled corn per plant. The vigorous plants tasseled on the average about nine days earlier than the weak plants.

From time to time various investigators have attributed the cause of slow and irregular growth in maize to the influence of pathogenic organisms. Burrill (3) in discussing a bacterial disease of corn said:

The first indication of the disease in a field of corn, as noticed in ordinary observation, is the dwarfed condition of the young plants.

Duncanson (8) noted that diseased plants were dwarfed, slender, and generally unhealthy. Stewart (37), working with sweet corn (*Zea mays saccharata*), noted that—

usually, the small plants are the first to succumb to the disease, which fact suggests that the disease may be the cause of their slow growth. This suspicion was confirmed by microscopic examination. Plants green and apparently healthy except for their small size, were found to contain a considerable quantity of the bacterium in their vessels, while in the larger, more vigorous plants, the bacterium could not be found. However, in wet weather the bacterium may sometimes be found in quite vigorous plants.

Selby (34) noted conditions similar to those described by Burrill. Later he (35) stated that the root rot of corn caused a dwarfing of the plants and a failure of the stalk to produce an ear. Hoffer and Holbert (15) pointed out that the use of diseased seed corn was one of the causes of high percentages of blighted and stunted plants and subsequent unsatisfactory yields. Rosen (32), in describing a bacterial disease of corn, noted that the diseased plants could be recognized easily by the short, stunted nature of the stalks. Holbert, Dickson, and Biggar (16) conducted experiments in which half of the hills were inoculated at planting time with a pure culture of *Gibberella saubinetii* (Mont.) Sacc. They summarized their results as follows:

In the inoculated hills germination was lowered, early growth retarded, relative vigor during the season reduced, and grain production lessened.

The pathogene with which they were inoculated was readily recovered from the diseased plants during the seedling stage. They stated further that—

Plants, strong or weak in the early stages of growth, had a tendency to retain this relative vigor throughout the season. Average grain production was directly correlated with rate of early growth and early vigor.

Trost and Hoffer (38) found that starchy ears of corn of certain dent varieties produced larger numbers of weak-growing and root rot susceptible plants in the field and yielded less per acre than the plants from ears of more horny composition, irrespective of whether or not the starchy kernels were infected with *Fusarium* spp. before planting.

Baker (2), Butler (4), Palm (29), Rutgers (33), and others working in the Orient have found that the downy mildews (*Sclerospora* spp.) in attacking young maize plants, cause a serious stunting in their early growth. Plants so affected seldom produce grain. A complete description of this disease is given by Weston (39).

## EXPERIMENTAL METHODS

The investigations here reported include data on approximately 153,000 corn plants grown (1) in experimental plots in the vicinity of Bloomington, Ill., in the four years 1918 to 1921, on uniform, well-drained, fertile soil, classified by the Illinois Soil Survey (18) as brown silt loam; (2) on the Agronomy South Farm of the Illinois Agricultural Experiment Station, University of Illinois, Urbana, a brown silt loam (19); and (3) on an experimental plot near Yates City, Ill., also a brown silt loam (20). The seed, representing varieties differing in length of growing season, was all selected before a killing frost, carefully dried, and properly stored.

The term "relatively disease-free," wherever used in the tables, refers to seed showing no symptoms of infections by any of the root and stalk rot pathogenes, including species of *Gibberella*, *Fusarium*, and *Diplodia*. This information was obtained by testing representative samples on the limestone-sawdust table germinator described by Holbert and Hoffer (17). The term "diseased" refers to seed showing such infections when tested by this same method. The testing of every kernel of a great number of seed ears has given abundant evidence that usually not all the kernels on ears classed as "relatively disease-free" are actually free from infections. Nor is every kernel on ears classed as diseased actually infected; in fact, the percentage of infected kernels in any of the diseased seed lots used usually was less than 50. The term "nearly disease-free" refers to seed lots as nearly free from infection as it was possible to have them.

The plots were hand planted in hills 42 inches apart each way at the rate of three kernels per hill. Great care was exercised throughout the season to avoid mechanical injury of the plants during cultivation and to guard against ravages of insect pests and rodents, but no attempt was made either to thin to a uniform stand or to correct for differences in stand. When there was any appreciable damage due to these causes the experiments were discarded entirely. Both acre yields and individual plant yields were recorded in terms of the weight of dry shelled corn. Acre yields have been reduced to a uniform moisture basis.

Measurements and other readings taken in connection with these investigations were not made on plants selected at random; every individual plant in each of the various experiments herein reported was measured and the measurements were included in the data. Plant height was measured to the tip of the tallest leaf. Stalk circumference was measured at the middle of the first internode above the ground surface.

Each plant was studied in the early growth stages from the standpoint of vigor and classified as either vigorous, semivigorous, or weak. This classification was based on the general appearance of the plant, including size, color of foliage, apparent freedom from disease symptoms, and other characters usually associated with vigor. With few exceptions these records were made about 30 days after planting.

For three years corn inoculation studies have been conducted in cooperation with Dr. James G. Dickson, of the Office of Cereal Investigations, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station, to investigate further the relation of *Gibberella saubinetii*, the common wheat-scab organism (23), to the root and stalk rots of corn. It has been found that this organism, under conditions to be discussed in other papers, may be very active in reducing early vigor of corn plants. A limited number of these data are included in this paper to show

that reduced early vigor, as a result of artificial inoculation with one of the root and stalk rot pathogenes, also is directly correlated with reduced yield. The term "inoculated," as used in the tables, refers to corn grown from seed inoculated at planting time with *Gibberella saubinetii*, the inocula being prepared by Dickson. The term "uninoculated" refers to corn in contiguous plots of equal size grown from similar seed not inoculated.

The yields from experiments conducted in 1920 and 1921 were separated into "marketable" and "unmarketable" grades. The latter grade consisted of nubbins, rotted or partly rotted ears, and light chaffy ears. Ears that were less than half the length of an average large-sized ear of the variety were classed as nubbins. Larger ears also that were poorly filled were classed as nubbins. The term "rotted ears" included any that showed an appreciable extent of rot due to *Diplodia*, *Fusarium*, or other causes. The chaffy ears were those with the grains imperfectly matured so that the kernels were thin and loose on the cob. Chaffy ears that also showed signs of rot were classed as rotted. (See Pl. 2, 3, and 4.)

The term "infested" soil, as used in this paper, refers to soil known to be infested with one or more of the root and stalk rot pathogenes. In every case where this term is used the soil had been cropped to corn or wheat for the previous three years. The wheat had been heavily infected with scab (*Gibberella saubinetii*), and the corn crops were known to be badly diseased.

The term "clean" soil, as used in this paper, refers to soil reasonably free from any of the root and stalk rot pathogenes. This soil had not been cropped to corn or wheat for at least 10 years.

#### • EXPERIMENTAL DATA

The experimental data cover numerous and extensive studies on the relation of the vigor of corn plants in the early stages of their growth to their subsequent yield, under different conditions. The variables include differences in infection of the seed by rot-producing pathogenes, differences in physical character of the kernels, and differences in the soil, in character, treatment, and quantity of infestation.

#### COMPARISON OF EARLY HEIGHT AND YIELD OF TRANSPLANTED SEEDLINGS

During the summer of 1918 a number of corn seedlings were transplanted to the field and studied in order to compare the performance of apparently disease-free and diseased seedlings. The seedlings were grown on the limestone-sawdust table germinator until the plumules were approximately 3 inches long. At this time those selected for further study were transferred to small paper boxes containing moist soil free from any of the corn rot pathogenes. One day later the boxes and seedlings were transferred to the field, the healthy and diseased seedlings being placed in alternate hills 42 inches apart. Both types of seedlings used in these studies had the same height and relative vigor when taken from the germinator. These seedlings were so selected that they represented adjacent kernels on artificially cross-pollinated ears from apparently healthy plants. The field in which these studies were carried on had not grown a crop of corn for at least 10 years.

The healthy seedlings suffered no ill effect from transplanting and made steady growth in height from the time they were transplanted.

On the other hand, all of those in the diseased group remained stationary in height for a period of 10 to 15 days. At the end of that time some of the diseased plants began growing and continued to progress at about

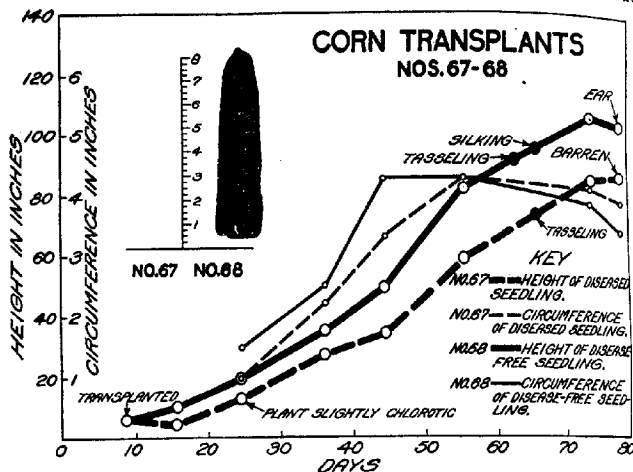


FIG. 1.—Growth curves and yield of diseased (No. 67) and healthy (No. 68) corn transplants. During the first week there was an actual decrease in height of the diseased transplant on account of the blighting of the leaves. During the following three weeks the diseased plant recovered somewhat from the severe attack of the root and stalk rot pathogens and grew at about the same rate as the healthy plant, but after that, it failed to make as rapid progress. The diseased transplant required about 10 days longer to attain its maximum circumference. It failed to produce any grain, whereas the healthy transplant produced the good ear shown.

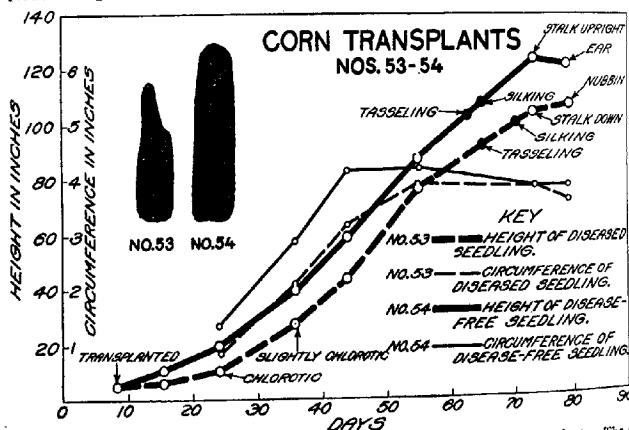


FIG. 2.—Growth curves and yield of diseased (No. 53) and healthy (No. 54) corn transplants. The diseased transplant failed to make any progress in height during the first week or 10 days, after which it grew at a rate parallel with that of the healthy transplant. The diseased transplant was considerably later in attaining its maximum stalk circumference, in tasseling, and in silking and produced a considerably smaller ear.

the same rate as the healthy plants with which they were compared (fig. 1 and 2). Some of the diseased plants finally attained a height equal to that of the healthy plants (fig. 3) but they were later in tasseling

and silking and did not yield nearly so much grain as the healthy plants. Others, however, grew very slowly and finally succumbed to the attacks of the parasitic organisms with which they were infected.

A summary of these data is given in Table I. It will be observed that the diseased transplants never recovered sufficiently from the stunting in growth during the first 15 days to produce a normal yield of grain. In some cases the diseased plants bore a cob of normal length but on it there were only a few kernels.

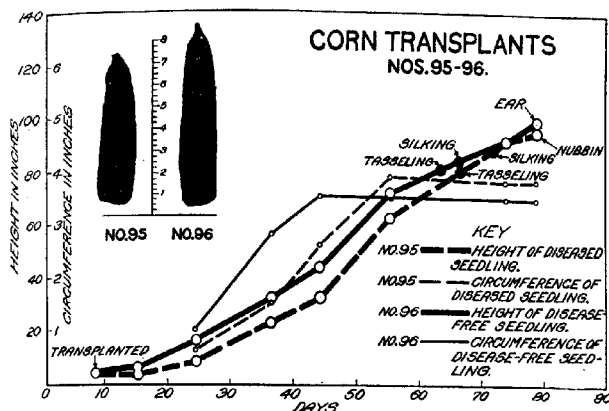


FIG. 3.—Growth curves and yield of diseased (No. 95) and healthy (No. 96) corn transplants. The greatest difference in height and stalk circumference occurred during the interval between 25 and 45 days after planting. After that time the diseased transplant seemed gradually to recover as far as size was concerned. This apparent recovery, however, was of no significance, for it produced only a short ear which was badly rotted, whereas the healthy transplant produced a sound ear of normal size.

TABLE I.—Comparative field performance of 34 healthy and 34 diseased transplants of Funk Ninety-Day corn grown in clean soil, at Bloomington, Ill., in 1918

Points considered.	Condition of seedlings when transplanted.	
	Apparently disease-free.	Diseased.
Number of seedlings:		
Transplanted.....	34	34
Dying within 16 days after transplanting.....	0	16
Living throughout season.....	34	18
Number of transplants producing:		
Two ears.....	7	1
One well-developed ear.....	18	4
One mid-sized ear.....	3	5
One nubbin only.....	3	4
Number of barren plants.....	3	4

#### CORRELATION OF EARLY HEIGHT AND YIELD OF FIELD-GROWN PLANTS

Table II gives the average heights, average yields, and correlation coefficients of height and yield of the plants in two contiguous plots of corn.



The plant measurements were taken 35 days, 56 days, and 146 days after planting.

TABLE II.—Correlation of height (determined at different dates after planting) and yield, in Reid Yellow Dent grown from relatively disease-free and diseased seed in infested soil, at Bloomington, Ill., in 1918

Condition of seed.	Number of plants.	Days after planting.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
			Cm.	Gm.	°	°
Relative disease-free....	157	35	91.1	201.1	+0.512	±0.046
	157	56	187.3	201.1	+ .512	± .046
	157	146	281.9	201.1	+ .319	± .056
Diseased.....	119	35	86.1	181.2	+ .474	± .042
	119	56	189.1	181.2	+ .455	± .043
	119	146	275.1	181.2	+ .502	± .037

The correlation coefficients in this table range from nearly 6 times to nearly 15 times the probable error. Love, of the Plant Breeding Department of Cornell University, and others, express the opinion that correlation coefficients which are more than 3.8 times the probable error are somewhat significant. King, as quoted by Babcock and Clausen (1), gives the following rules for the interpretation of the coefficient of correlation according to its relation to the probable error:

1. If the correlation coefficient is less than the probable error, there is no evidence whatever of correlation.
2. If the correlation coefficient is more than six times the size of the probable error, the existence of correlation is a practical certainty.
3. In cases where the probable error is relatively small:
  - (a) If the correlation coefficient is less than 0.3 the correlation can not be considered at all marked.
  - (b) If the correlation coefficient is above 0.5 there is a decided correlation.

Table III gives correlation coefficients between stalk circumference and yield of the same plants studied in Table II and presents the only correlations between circumference and yield which appear in this paper. They are given at this time in connection with the height data of the same plants to show that the two characters are seemingly well correlated.

TABLE III.—Correlation of circumference of stalk (measured at different dates after planting) and yield in Reid Yellow Dent, grown from relatively disease-free and diseased seed on infested soil, at Bloomington, Ill., in 1918

Condition of seed.	Number of plants.	Days after planting.	Average circumference.	Average yield per plant.	Correlation coefficient.	Probable error.
			Cm.	Gm.		
Relatively disease-free..	157	35	5.6	201.1	+0.618	±0.038
	157	56	9.4	201.1	+ .478	± .088
	157	146	9.0	201.1	+ .643	± .036
Diseased.....	119	35	5.3	181.2	+ .643	± .036
	119	56	9.8	181.2	+ .568	± .036
	119	146	9.2	181.2	+ .620	± .033

TABLE IV.—*Correlation of early height (36 days after planting) and yield in Funk Ninety-Day corn grown on clean soil from relatively disease-free and diseased seed, at Bloomington, Ill., in 1919*

Condition of seed.	Number of plants.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
		<i>Cm.</i>	<i>Gm.</i>		
Disease-free.....	354	150.4	191.0	+0.359	±0.031
Diseased.....	331	146.3	179.4	+0.435	±0.030
Seed selected from normally ripened plants.....	243	156.3	212.6	+0.305	±0.039
Seed selected from prematurely ripened plants.....	244	154.0	197.0	+0.307	±0.039

The experimental plots represented in Table IV were not planted until the latter part of the normal corn planting season. The soil in these plots had not grown corn during the previous 10 years. These facts probably account for the correlation coefficients being lower than those given in Table II. Yet it seems that, even under these conditions, the correlation between early height and yield is significant.

TABLE V.—*Correlation of height and yield in Bloody Butcher corn grown from artificially pollinated ears. Seed planted May 30 in infested soil at Bloomington, Ill., in 1919*

Group No.	Condition of parent plant.	Treatment of seed.	Number of plants.	Days after planting.	Average height.	Average yield per plant.	Correlation coefficient.	Probable error.
					<i>Cm.</i>	<i>Gm.</i>		
I	Diseased plants selfed.	Uninoculated....	265	30	67	132	+0.167	±0.040
		Inoculated....	200	30	57	123	+0.360	±0.041
II	Apparently disease-free plants crossed	Uninoculated....	240	30	75	218	+0.405	±0.037
		Inoculated....	208	30	72	240	+0.366	±0.045
		Uninoculated....	240	52	213	218	+0.569	±0.029
		Inoculated....	208	52	211	240	+0.429	±0.038
III	Apparently disease-free plants selfed.	Uninoculated....	72	30	56	254	+0.432	±0.064
		Inoculated....	57	30	43	233	+0.537	±0.063
		Uninoculated....	72	52	210	254	+0.600	±0.051
		Inoculated....	57	52	199	233	+0.680	±0.048
IV	Diseased plants crossed.	Uninoculated....	65	30	92	155	+0.447	±0.067
		Inoculated....	50	30	93	113	+0.007	±0.095
		Uninoculated....	65	52	211	155	+0.650	±0.048
		Inoculated....	50	52	204	113	+0.205	±0.085

In each group shown in Table V, series of plantings of seed inoculated with *Gibberella saubinetii* were made along with corresponding series with uninoculated seed.

There seems to be no special relation between the correlation coefficients and inoculation in the four different groups. However, there is in most cases a strong correlation between early height and yield. The correlation coefficients are higher for the measurements taken 52 days after planting than for those taken 30 days after planting. This can be accounted for by the fact that weak plants make slower gains in height during that period of their development (Tables VIII and IX). It will be noted that the inoculation (*Gibberella saubinetii*) materially reduced

the stand in every comparison and the average yield per plant in every one except group 2. In this group there was evidence of resistance, which probably accounts for the different response of the plants in this group to the inoculation.

#### RELATION OF <sup>1</sup>EARLY VIGOR AND YIELD AS INFLUENCED BY SEED INFECTION

##### STUDIES OF INDIVIDUAL PLANTS

General observations, growth records, and yield data on the experimental plots planted with apparently disease-free and diseased seed impressed the authors with the fact that the recorded differences in stand did not accurately represent the actual differences between the various plots of corn in their early stages of growth. The classification of the plants as vigorous, semivigorous, or weak was an attempt to record more accurately the comparative condition of the plots at that time. Although this classification may be subject to personal error at times, it has been used advantageously in expressing significant differences in early vigor.

When this classification was first used a number of plants were selected for study about whose classification as vigorous, semivigorous, or weak, there was no doubt (Pl. 1 and 5). The plants were harvested individually and the dry-shelled weight of each ear determined. These data are presented in Table VI, where one may note the strong correlation existing between early vigor and yield, both in average production and in absence of barren stalks. Occasionally, however, plants classified as vigorous in the juvenile stage either may be barren or produce nubbins only; but this is the exception rather than the rule. At least one important disease of corn that does not affect early vigor appreciably appears to cause a certain amount of barrenness and nubbins production. This phase of the corn, root and stalk rot problem will be discussed in another paper.

TABLE VI.—*Relation of early vigor to yield and barrenness in plants of Reid Yellow Dent, classified as vigorous, semivigorous, or weak, 35 days after planting, at Bloomington, Ill., in 1918*

Points considered.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants.....	601	259	250
Percentage of plants dying.....	3.3	3.2	9.8
Average yield per plant (in grams).....	249.4	139.9	100.5
Percentage of barren plants.....	6.5	32.8	50.0

Table VII gives data on an experiment conducted with disease-free and diseased seed of Funk Ninety-Day corn. The plot was divided and one-half was planted with each kind of seed. A glance at the percentage of vigorous and weak plants grown from relatively disease-free seed as compared with the plants grown from diseased seed shows that the percentage of vigorous plants drops from 64.3 in the disease-free half to 29.1 in the diseased half, while the percentage of weak plants rises from 8.8 to 19.1. Early vigor and average yield of grain per plant, as well as absence of barren stalks, are directly correlated.

TABLE VII.—Relation of early vigor 30 days after planting to yield, as influenced by seed infection in Funk Ninety-Day corn, grown from relatively disease-free and diseased seed on infested soil, at Bloomington, Ill., in 1918

Points considered.	Relatively disease-free seed.			Diseased seed.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants..	139	58	19	55	98	36
Percentage of plants in each group.....	64.3	26.9	8.8	29.1	51.8	19.1
Average yield per plant (in grams).....	243.5	207.2	97.7	241.5	172.7	86.8
Percentage of barren plants.....	.1	3.5	26.3	0	7.2	50.0

TABLE VIII.—Relation of early vigor to rate of growth and yield as influenced by seed infection in Funk Ninety-Day corn grown on clean soil at Bloomington, Ill., in 1919

Points considered.	Relatively disease-free seed.			Slightly diseased seed.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants..	146	159	45	123	165	38
Percentage of plants in each group.....	41.8	45.6	12.8	37.7	50.6	11.7
Average height (in centimeters) 15 days after planting.....	44.3	37.7	26.8	43.3	36.2	24.4
Average height (in centimeters) 36 days after planting.....	158.0	146.6	123.8	158.8	143.4	114.5
Average increase in height per plant (in centimeters) 21 days after first record.....	113.7	108.9	97.0	115.5	107.2	90.1
Average yield per plant (in grams).....	199.9	192.0	158.0	184.6	176.6	135.8

TABLE IX.—*Relation of early vigor to rate of growth and yield of Funk's Ninety-Day corn as influenced by pathologic condition of plants from which seed was selected, grown on clean soil at Bloomington, Ill., in 1919*

Points considered.	Seed from ears ripening normally.			Seed from ears ripening prematurely.		
	Vigorous.	Semi-vigorous.	Weak.	Vigorous.	Semi-vigorous.	Weak.
Total number of plants..	78	138	27	61	156	27
Percentage of plants in each group.....	32.1	56.8	11.1	25.0	63.9	11.1
Average height (in centimeters) 15 days after planting.....	45.7	38.5	27.4	44.4	36.8	28.8
Average height (in centimeters) 36 days after planting.....	169.0	154.2	126.6	170.1	150.3	130.4
Average increase in height per plant (in centimeters) 21 days after first record.....	123.3	115.7	99.2	125.7	114.5	101.6
Average yield per plant (in grams).....	220.5	215.4	177.8	220.2	194.8	147.1

Tables VIII and IX are of special interest because of the fact that the classification of plants as vigorous, semivigorous, and weak was made only 15 days after the corn was planted and because the corn was planted at the end of the normal corn planting season in soil that had not grown corn for 12 years. These results lend further emphasis to the strong correlation of early vigor and yield.

In the experiments reported in Tables VIII and IX, the heights of the plants were taken 15 days after planting at the time of judging vigor, and again at 36 days after planting. In both measurements there is a fairly wide variance in the average heights of vigorous, semivigorous, and weak plants. In the period of 21 days between the first and second measurements all groups of plants made considerable gains in heights. A comparison of the average gains in plant height during this period shows that, in every comparison, the vigorous plants made the greatest gains, followed by the semivigorous plants, and then by the weak. It is evident that the more vigorous individuals not only retain their initial superiority in height but grow more rapidly than the less vigorous plants. The difference between the vigorous and weak plants in this respect is very marked.

#### STUDIES OF PLANT POPULATIONS

From the foregoing data and discussion it seems evident (1) that early vigor and yield of grain are closely correlated (Pl. 4), also (2) that the percentages of strong, vigorous plants in populations grown from diseased seed are lower than in similar populations grown from relatively disease-free seed (Pl. 6 and 7). Inasmuch as the total yield of a plot or field of maize depends on the total number of plants and the average yield per plant, it follows that the total yields of contiguous plots, differing from 0 to 8 per cent in field stand, will be more or less directly proportional to the percentages of strong, vigorous plants in the populations. A large number of experiments have been conducted during a

period of four years in which alternate plots were planted with relatively disease-free and diseased seed. Where field stands and other factors were reasonably comparable, there has been a reduction in the percentage of vigorous plants, as classified 25 to 35 days after planting, as well as a reduction in final acre yield in bushels, although the relation varied from year to year, depending on seasonal conditions. These data are summarized in Table 10. The 1920 data are presented in more detail and discussed later in this paper. A part of the 1921 data is presented in both detailed and summarized forms to show more clearly the methods used in the investigations on which this paper is based.

TABLE X.—Summary of data showing influence of seed infection on early vigor and yield of corn grown from relatively disease-free and diseased seed, at Bloomington, Ill., in 1918, 1919, 1920, and 1921

Year.	Number of experiments.	Total number of plants classified.	Average reduction in percentage of vigorous plants.	Average increase in percentage of weak plants.	Average decrease in acre yield.
					Bushels.
1918.....	7	22,479	9.7	4.8	14.4
1919.....	7	29,013	13.3	6.3	12.9
1920.....	8	9,318	15.4	4.2	8.6
1921.....	4	6,134	22.6	4.9	9.2

#### RELATION OF EARLY VIGOR AND YIELD AS INFLUENCED BY PATHOLOGIC CONDITION OF MOTHER PLANT

In the fall of 1917, attractive ears were selected from vigorous and apparently healthy plants and from plants whose roots and stalks were rotted. The seed was planted the following spring in ear rows, the seed from apparently healthy plants being alternated with the seed from diseased plants. Similar experiments were conducted during the following two years. The data from these experiments are given in Table XI. Seed ears selected from plants whose roots and stems are badly rotted may or may not be infected with the rot pathogenes. In many cases they are not infected to such an extent that the infection can be detected by a careful germination test. But when these ears are used for seed they usually produce plants that are very susceptible to the root and stalk rots.

TABLE XI.—Early vigor and yield as influenced by the pathologic condition of mother plants from which seed was selected. The table shows reduced vigor and reduced yield in plants grown from seed selected from diseased plants, at Bloomington, Ill.

Year.	Variety.	Number of plants.	Reduction in stand.	Reduction in vigorous plants.	Increase in weak plants.	Decrease in acre yield.	Reduction in yield.
			Per cent.	Per cent.	Per cent.	Bushels.	Per cent.
1918	Reid Yellow Dent.....	5,140	10.9	4.4	1.2	7.3	10.9
1918	Funk 90-Day.....	6,617	.5	14.1	2.8	11.4	16.3
1919	do.....	2,090	2.1	15.4	1.3	6.8	9.2
1919	do.....	927	1.5	5.5	1.6	2.5	4.3
1920	Reid Yellow Dent.....	1,357	5.2	9.5	1.0	12.4	16.5
1920	do.....	3,144	.1	4.7	1.2	6.0	7.5

A glance at Table XI shows that seed selected from diseased mother plants produces plants deficient in early vigor and lacking in the ability to produce high grain yields. This is especially pronounced when the seed is planted in infested soil.

Seed ears from badly diseased plants usually are lacking in luster, and physical examination of the kernels on such ears frequently shows them to be more or less starchy (21). Specific gravity determinations on these kernels also reveal a tendency toward decreased density. Table XII gives further data regarding the comparative field performance of starchy and slightly starchy seed, as compared with the harder, more horny seed. The field stands and seed infections were comparable in all cases. Ordinarily, however, as Trost and Hoffer (38) have reported, ears with starchy kernels are very often infected with one or more of the corn rot pathogens.

TABLE XII.—*Influence of endosperm composition (physical determination) on early vigor and yield. Data show reduced vigor and reduced yield in plats planted with starchy and slightly starchy seed as compared with similar plots grown from horny seed at Bloomington, Ill.*

Year.	Condition of seed.	Total number of plants.	Reduction in vigorous plants.	Increase in weak plants.	Reduction in acre yield.	Reduction in acre yield.
			Per cent.	Per cent.	Bushels.	Per cent.
1918	Starchy.....	1, 273	14.3	2.7	13.5	19.7
1918	Slightly starchy.....	9, 243	7.7	3.0	9.9	14.5
1919	Starchy (individual plants).....	170	14.0	4.0	.....	8.7
1920	Slightly starchy.....	926	21.7	1.0	4.8	9.0

#### RELATION OF EARLY VIGOR TO SIZE OF EARS PRODUCED

As might be expected from the data presented up to this point, early vigor and size of ears are strongly correlated. Plants vigorous and apparently healthy in their early stages of growth are much more likely to produce good-sized and well-matured ears than plants stunted during this same period. This fact is brought out clearly in Table XIII.

TABLE XIII.—*Relation of early vigor to the size of ears on 1,715 individual corn plants grown at Bloomington, Ill. in 1918*

Variety and condition of seed.	Percentage of plants classified in their early stages of growth as vigorous, semivigorous, and weak which produced good-sized, well-matured ears.		
	Vigorous.	Semivigorous.	Weak.
Reid Yellow Dent.....	67.5	18.9	15.1
Funk Ninety-Day (relatively disease-free).....	92.0	53.4	0
Funk Ninety-Day (diseased seed).....	83.6	54.1	2.6

#### RELATION OF EARLY VIGOR TO SHRINKAGE OF GRAIN

It has been found, as East and Hayes (11) and Grantham (14) have reported, and as many other investigators have observed, that weak plants are from 7 to 10 days later in tasseling and silking than vigorous

plants. Observations and records on many hundreds of individual plants at frequent intervals over a period of four years have indicated that plants stunted in their early growth as a result of attacks by the root and stalk rot pathogenes are nearly always somewhat later in tasseling and silking (figures 1, 2, and 3). These plants, therefore, on account of delayed pollination and fertilization combined with their weakened condi-

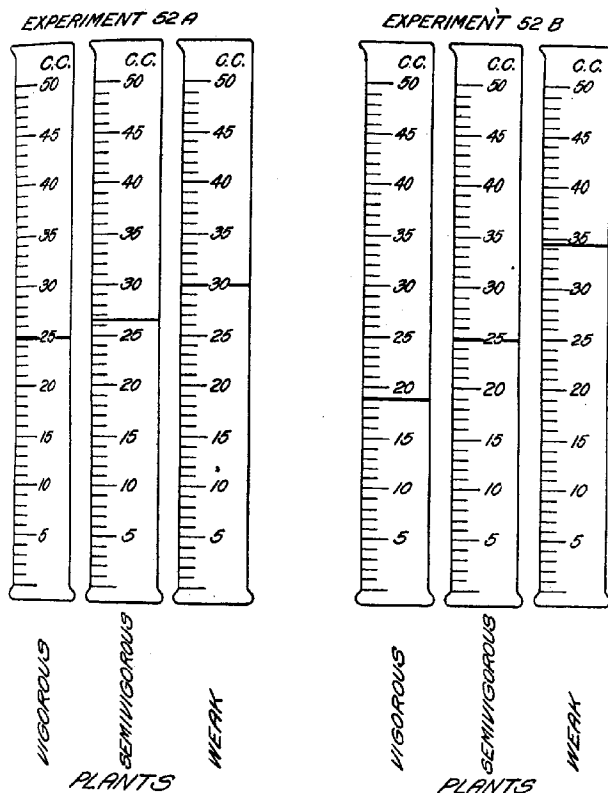


FIG. 4.—Graph showing cubic centimeters of water lost in the curing of 100 gm. of freshly harvested corn produced on vigorous, semivigorous, and weak plants, grown from uninoculated seed. (See Table XIV.)

tion, can not mature their grain as early nor as completely as the more nearly normal plants. As a result, the corn from the weak plants and from diseased plants usually contains more moisture when harvested and consequently shrinks more when reduced to an air-dry condition. Some data on this are shown in Table XIV.



TABLE XIV.—Relation of early vigor to shrinkage in weight of grain, determined on 1,325 plants of Funk's Ninety-Day corn in two inoculation experiments, alternate hills of which were inoculated at planting time with *Gibberella saubinetii* grown on clean soil of high fertility at Bloomington, Ill., in 1919

Experiment No. <sup>1</sup>	Treatment of seed.	Shrinkage of grain from plants classified in their early growth stages as vigorous, semivigorous, or weak.		
		Vigorous.	Semivigorous.	Weak.
		Per cent.	Per cent.	Per cent.
52 A. ....	{Uninoculated.....	24.7	26.4	30.1
	{Inoculated.....	26.4	27.3	27.2
52 B. ....	{Uninoculated.....	18.9	24.8	34.4
	{Inoculated.....	21.5	28.4	29.2

Table XIV gives the percentage of shrinkage of the grain from vigorous, semivigorous, and weak plants harvested only a few days before a killing frost. The product from each plant was weighed separately when harvested and again after the ears were thoroughly air-dried. The results show that in every case there is a greater percentage of shrinkage in the grain produced by the weak than by the vigorous plants (fig. 4). Plants of medium vigor tend to have an intermediate percentage of shrinkage. This indicates that the same factors which enable the more vigorous plants to grow most rapidly and to excel in grain production also cause the grain to mature earlier. At harvest time there was little apparent difference in the height of plants that were classified in their early stages of growth as vigorous, semivigorous, and weak. Incidentally it is of interest to note that there is a slightly greater shrinkage in the product of the inoculated rows than in that of the uninoculated in the vigorous and semivigorous groups. In the groups of weak plants the situation is reversed, the weak plants having a smaller percentage of shrinkage in the inoculated than in the uninoculated rows. This may be accounted for by the fact that many of the weak plants in the inoculated rows died prematurely, thus permitting partial curing before harvest.

#### RELATION OF EARLY VIGOR AND QUALITY OF PRODUCT AS INFLUENCED BY SEED INFECTION

DATA OBTAINED IN 1920

In addition to the reduced size of ear and reduced yield of shelled grain from plants semivigorous or weak in early vigor as compared with the yield of plants strong and apparently healthy in their early stages of growth, there is a distinct difference in the quality of the grain. Because many of the less vigorous and diseased plants are killed by frost before the grain is mature, there are more rotted ears and more light chaffy ears in the corn produced from semivigorous and weak plants than from vigorous plants under comparable conditions. As brought out in previous tables, corn grown from diseased seed contains fewer strong plants and more weak plants than corn grown from relatively disease-free seed. Consequently, it would be expected that corn grown from diseased seed would contain more nubbins, more rotted ears, and more light, chaffy ears.

than corn grown from nearly disease-free seed. Much of this corn of inferior quality would be left in the field unharvested by most farmers. Many farmers who might gather such corn from the field probably would either sort it out when dumping the corn at the crib or when shelling it for market. At any rate, small nubbins, rotted ears, and chaffy ears represent corn of low grade and low selling value.

In view of these facts it was deemed advisable, beginning in 1920, to report separate yields of marketable and unmarketable corn. Plates 2, 3, and 4 illustrate the kinds of corn that were classified as marketable and unmarketable. There are occasional ears about whose classification there might be some question; but, on the whole, personal judgment changes but little within a given experiment, and errors due to personal factors are comparatively few when a large number of experiments are concerned. Although this procedure is imperfect, it gives a more accurate index to the value of any yield of corn than total yield alone. When considering yield under actual farm conditions, quality should be as important as quantity, a fact forcibly emphasized in the 1921 data.

In presenting data from experiments conducted in 1920 and 1921, it seemed desirable to report yields in terms of percentages of increased yield of nearly disease-free over the moderately diseased seed, inasmuch as the moderately diseased seed used was representative of the majority of seed ears used by the farmers supplying the seed for the experiments. The yields from these different lots of moderately diseased seed were higher than the average corn yield of the counties in which the experiments were conducted. On the Agronomy South Farm of the Illinois Agricultural Experiment Station at Urbana, these lots of moderately diseased seed compared favorably in yield with the standard varieties. The nearly disease-free seed lots used in the experiments (1920 and 1921) were practically free from any of the root and stalk rot pathogenes, as evidenced by repeated laboratory determinations, and represented the best seed ears available in the open fertilized seed stocks of the Corn Belt.

TABLE XV.—Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn. Reid Yellow Dent (Macon County strain) grown on infested soil of medium fertility, at Bloomington, Ill., in 1920

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	Per cent.
Number of plants in experiment.....	727	846		
Early vigor:				
Percentage of kernels producing plants.....	75.8	88.1	16.2	
Percentage of kernels producing vigorous plants.....	29.2	45.3	55.2	
Percentage of kernels producing weak plants.....	12.3	6.3		48.8
Yield:				
Total acre yield (in bushels).....	49.8	63.5	27.5	
Acre yield of marketable corn.....	33.7	54.6	62.0	
Percentage of nubbins (by count).....	23.3	11.6		50.2
Percentage of rotted ears (by count).....	10.7	3.9		63.5
Percentage of chaffy ears (by count).....	26.0	7.7		70.4

Table XV gives data showing the influence of seed infection on early vigor and subsequent yield and quality. The increased percentage of

strong, vigorous plants in the plots planted with nearly disease-free seed is very marked (Pl. 6), as is the decrease in percentage of nubbins, rotted ears, and chaffy ears. The increase in yield of marketable corn is very significant. The relation of increased early vigor to increased yield of marketable corn is brought out more clearly in figure 5.

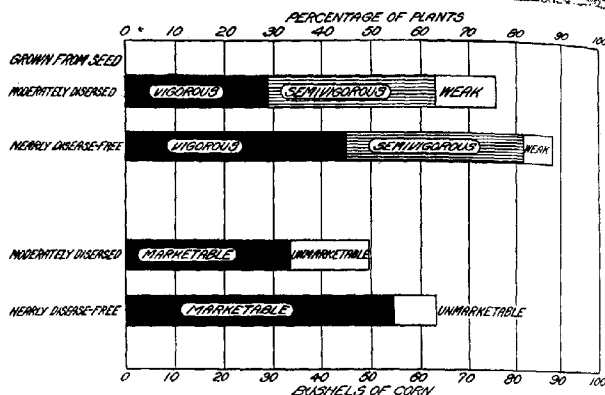


FIG. 5.—Graphic presentation of Table XV, showing relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

TABLE XVI.—Influence of moderately diseased and nearly disease-free seed of Funk strain of Reid Yellow Dent, on early vigor and yield of marketable corn, when grown on infested soil of medium fertility, at Bloomington, Ill., in 1920

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
Number of plants in experiment.....	323	361	Per cent.	Per cent.
Early vigor:				
Percentage of kernels producing plants.....	84.1	91.4	8.6	.....
Percentage of kernels producing vigorous plants.....	23.4	34.4	47.0	.....
Percentage of kernels producing weak plants.....	8.3	2.6	.....	68.7
Yield:				
Total acre yield (in bushels).....	57.3	61.7	7.6	.....
Acre yield of marketable corn (in bushels)...	42.1	54.7	29.9	.....
Percentage of nubbins (by count).....	11.8	8.2	.....	30.5
Percentage of rotted ears (by count).....	4.3	3.6	.....	19.4
Percentage of chaffy ears (by count).....	12.5	2.6	.....	79.2

Table XVI gives further data on the influence of seed infection on early vigor and subsequent yield. Figure 6 shows graphically the data given in Table XVI.

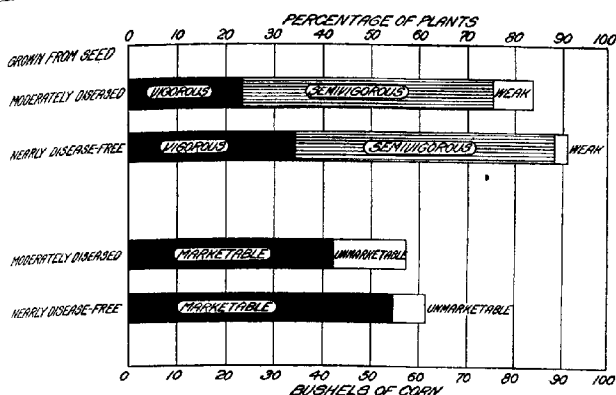


FIG. 6.—Graphic presentation of Table XVI, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

TABLE XVII.—Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of McKieghan strain of Reid Yellow Dent on soil of average fertility on which clover had been grown for the previous two years but which had previously never been limed nor phosphated (22), at Yates City, Ill., in 1920

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	Per cent.
Number of plants in experiment.....	1,082	1,251		
Early vigor:				
Percentage of kernels producing plants.....	75.1	86.8	15.6	
Percentage of kernels producing vigorous plants.....	29.4	39.1	33.0	
Percentage of kernels producing weak plants.....	16.2	13.1		19.1
Yield:				
Total acre yield (in bushels).....	67.0	72.4	8.1	
Acre yield of marketable corn (in bushels).....	44.5	52.4	17.8	

The increase in yield of marketable corn shown in Table XVII and figure 7 is not so marked as in previous tables, but it is large enough to be significant.

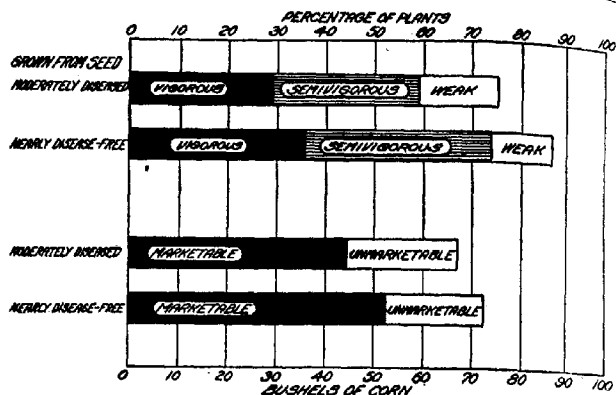


FIG. 7.—Graphic presentation of Table XVII, showing relation of diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

Tables XVIII, XIX, and XX give the results of similar experiments conducted on the Agronomy South Farm of the Illinois Agricultural Station at Urbana, Ill., during 1920, on very fertile soil (fig. 8), on soil of good average fertility, and on soil which has had 75 per cent of corn in the rotation for 13 years.

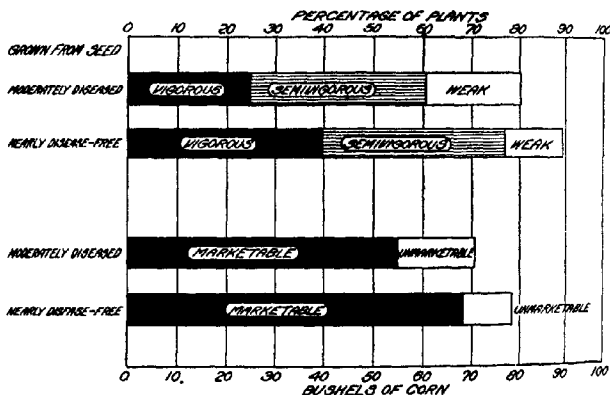


FIG. 8.—Graphic presentation of Table XVIII, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn, in bushels per acre.

In every case there has been a marked increase in the field stand, percentage of strong, vigorous plants, and yield of marketable corn in the plots grown from nearly disease-free seed. In Table XVIII the difference in stand might account for the difference in total yield, but the difference in yield of marketable corn must be accounted for in some other way. Figure 8 shows the relation of the increase in percentage of vigorous plants to the increase in yield of sound, marketable corn.

TABLE XVIII.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on very fertile soil to which rock phosphate and manure has been applied, at Urbana, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	Per cent.
Number of plants in experiment.....	3,850	4,281		
Early vigor:				
Percentage of kernels producing plants.....	80.3	89.3	11.2	
Percentage of kernels producing vigorous plants.....	25.2	39.4	56.3	
Percentage of kernels producing weak plants.....	19.5	12.3		36.9
Yield:				
Total acre yield (in bushels).....	70.7	78.7	11.3	
Acre yield marketable corn (in bushels).....	54.8	68.2	24.5	
Percentage of nubbins (by count).....	19.5	14.1		27.7
Percentage of rotted ears (by count).....	4.5	3.3		26.7
Percentage of chaffy ears (by count).....	8.5	4.3		49.4

The same standard for vigorous plants was maintained in taking the data reported in Tables XVIII, XIX, and XX. In the experiments reported in Table XX there was little apparent difference in vigor or general appearance in the early stages of growth between the rows grown from diseased and nearly disease-free seed. However, it was evident upon close examination that the plants in plots planted with nearly disease-free seed were somewhat larger in circumference at the base and also sturdier. The increase in yield from the better seed is very marked, even in heavily infested soil. Such results suggest that the nearly disease-free seed may have possessed some degree of resistance to the root and stalk rots.

TABLE XIX.—*Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on soil of average fertility, limed and phosphated, at Urbana, Ill., in 1920*

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	Per cent.
Number of plants in experiment.....	3,847	4,263		
Early vigor:				
Percentage of kernels producing plants.....	80.2	88.8	10.7	
Percentage of kernels producing vigorous plants.....	9.6	19.1	99.0	
Percentage of kernels producing weak plants.....	30.5	22.9		24.9
Yield:				
Total acre yield (in bushels).....	67.7	79.4	17.3	
Acre yield of marketable corn (in bushels)...	59.6	74.1	24.3	

TABLE XX.—Influence of moderately diseased and nearly disease-free seed on early vigor and yield of marketable corn of Reid Yellow Dent on heavily infested soil, limed and phosphated, at Urbana, Ill., in 1920

Points considered.	Moderately diseased composite.	Nearly disease-free composite.	In favor of disease-free seed.	
			Increase.	Decrease.
			Per cent.	Per cent.
Number of plants in experiment.....	2,096	2,338	.....	.....
Early vigor:				
Percentage of kernels producing plants....	74.5	83.4	11.9	.....
Percentage of kernels producing vigorous plants.....	1.7	2.6	52.9	.....
Percentage of kernels producing weak plants.	59.3	59.2	.....	0.2
Yield:				
Total acre yield (in bushels).....	39.2	47.9	22.2	.....
Acre yield of marketable corn (in bushels)...	32.4	41.3	27.5	.....

#### DATA OBTAINED IN 1921

The season of 1921 was different in many respects from the seasons of the previous three years. As a result of fungus injury following the unusually heavy attacks of the corn earworm (*Chloridea obsoleta* Fab.) during the warm, moist weather of the late summer and early fall, there was a very high percentage of rotted corn throughout the Corn Belt. In view of these facts the 1921 data relative to marketable and unmarketable corn in the yields from moderately diseased and nearly disease-free seed are of extraordinary significance. These data not only give further evidence on the important relations existing between seed infection, reduced early vigor, and reduced yields of sound corn, but show clearly that corn grown from seed infected with the root and stalk rot pathogenes, or seed susceptible to the root and stalk rots, is likely to be much more susceptible to fungi causing ear rots than corn grown from seed relatively free from these pathogenes. In most cases the different lots of corn were infested to approximately the same extent with earworms, but there was a vast difference in the degree to which the different lots were injured by the fungi following the attack of earworms. The principal fungi concerned with ear rots were *Diplodia zeae* and *Fusarium* spp.

The data presented in Tables XXI to XXV were secured from plantings on the no-treatment plots in a series of fertilizer experiments. These soil plots were not all equally fertile, but the soil variation within each plot probably was very slight. Consequently, comparisons should be made between the moderately diseased and nearly disease-free on each plot. The plantings on the various no-treatment plots were replications in which the same two seed composites were used throughout the four times of planting. The results are reported in Tables XXI to XXV and graphically presented in figures 9 to 13.

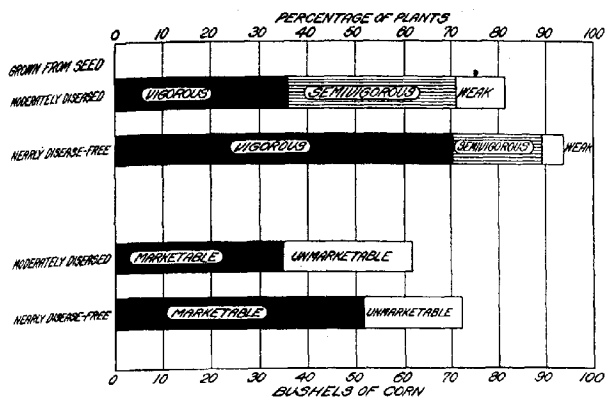


FIG. 9.—Graphic presentation of Table XXI, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

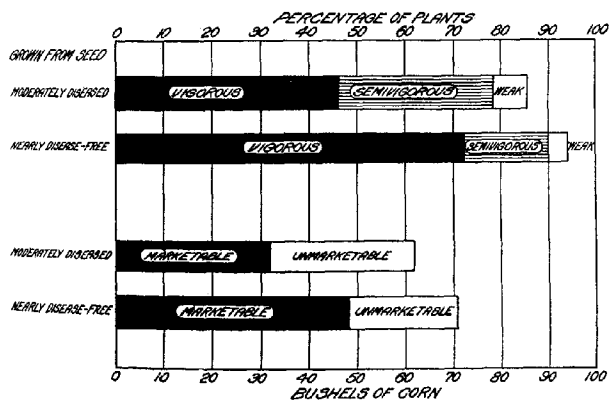


FIG. 10.—Graphic presentation of Table XXII, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.



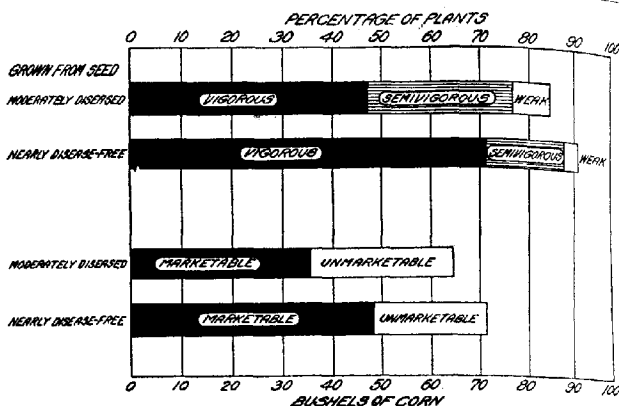


FIG. 11.—Graphic presentation of Table XXIII, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

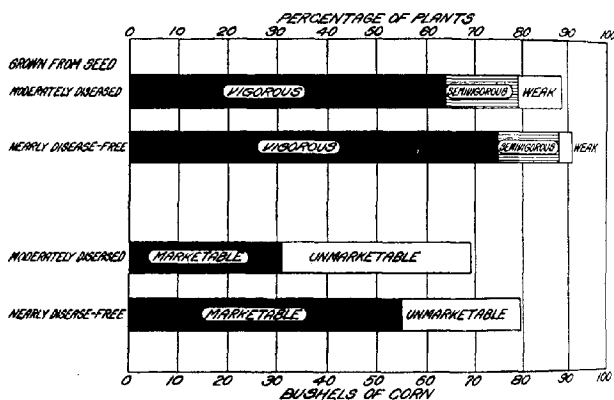


FIG. 12.—Graphic presentation of Table XXIV, showing the relation of moderately diseased and nearly disease-free seed to the percentage of vigorous, semivigorous, and weak plants produced, and to yield of marketable and unmarketable corn in bushels per acre.

The standard for vigorous plants in 1921 was somewhat different from that used during the previous three years of the investigations. On this account percentages of vigorous plants in 1920 can not be compared with percentages of vigorous plants in similar composites in 1921. However, the standards for vigorous, semivigorous, and weak plants were reasonably constant throughout each season, especially within any given experiment.

The soil was cold at the time the first planting was made and continued rather cold for 8 to 10 days. In this connection it is interesting to note that the field stand of the corn grown from moderately diseased seed is 12.2 per cent lower than that grown from the nearly disease-free composite (see Table XXI). By referring to Table XXV and figure 14 it

may be observed that the first planting showed the greatest difference in field stand between the moderately diseased and nearly disease-free composites.

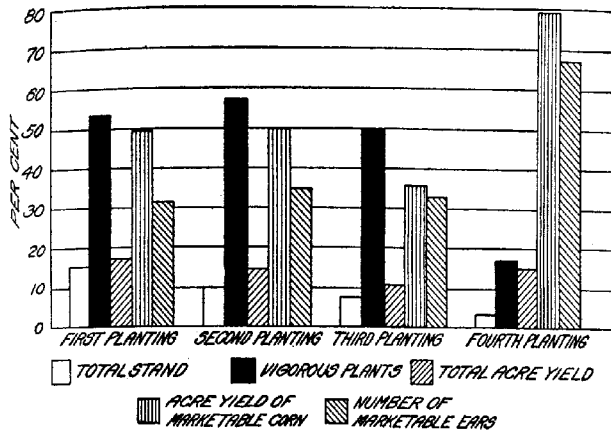


FIG. 13.—Graphic presentation of Table XXV, showing percentage increases in total stand, vigorous plants, total acre yield, acre yield of marketable corn, and number of marketable ears in favor of nearly disease-free seed over moderately diseased seed.

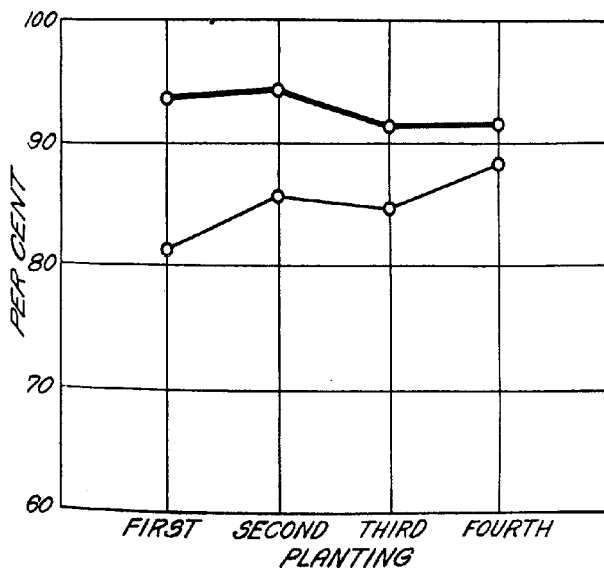


FIG. 14.—Graphic presentation of the percentage of field stand produced by the two lots of seed when planted at different dates, the heavy line representing nearly disease-free seed, the light line the moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)



Percentage increase in seed yield over mod- erately diseased seed	15.0	53.4	17.2	49.3	31.6	42.5	30.7
Percentage decrease of healthy disease- moderately dis- eased seed		57.3			15.3		

■ 99.7 per cent viable and 69.8 per cent diseased.      b 100 per cent viable and 3.3 per cent diseased.

TABLE XXII.—Comprehensive study of the principal data showing the influence of moderately diseased and nearly disease-free seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. Second planting (May 14) of the same series shown in Tables XXI, XXIII, and XXIV. Bloomington, Ill., 1921

Soil plot No.	Condition of seed.	Early vigor data.				Yield data.										Sound nubbins.														
		Total stand.		Vigorous plants.		Weak plants.		Marketable corn.			Unmarketable corn.			Market-able ears.				Rotted ears and nubbins.		Chaffy ears and nubbins.										
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling per cent.	Moisture per cent.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Moisture per cent.	Weight of dry matter.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
1	Moderately diseased.	85	88.5	47	49.0	5	5.2	51.2	41.4	18.1	69.7	46.8	25.8	21.864	15.6	17.9	14.6	12.3	14.3	10.5	37	59.6	14	19.2	14	19.2	14	19.2		
2	Nearly disease-free.	87	90.6	54	56.3	3	3.1	52.0	42.4	18.5	71.0	46.8	27.8	24.066	14.6	17.9	14.6	12.3	14.3	10.5	42	59.6	6	8.0	6	8.0	6	8.0		
3	Moderately diseased.	83	83.3	50	51.3	4	4.2	43.7	34.9	20.1	77.8	46.8	15.3	13.962	17.2	11.6	10.6	10.6	10.6	10.6	13.7	34	39.9	25	38.5	12	18.4	4	6.2	
4	Nearly disease-free.	94	97.9	58	70.8	2	2.1	57.1	48.4	15.2	77.8	49.2	35.8	30.555	17.6	21.3	17.6	14.8	16.4	12.4	43	53.7	22	27.5	10	12.5	5	6.3		
5	Moderately diseased.	90	91.3	51	52.0	4	4.4	46.8	37.8	18.2	68.5	38.1	25.5	21.864	15.6	17.9	14.6	12.3	14.3	10.5	43	53.7	22	27.5	10	12.5	5	6.3		
6	Nearly disease-free.	90	91.3	51	52.0	4	4.4	46.8	37.8	18.2	68.5	38.1	25.5	21.864	15.6	17.9	14.6	12.3	14.3	10.5	43	53.7	22	27.5	10	12.5	5	6.3		
7	Moderately diseased.	78	81.3	38	39.6	0	0.4	43.8	34.3	24.0	38.5	38.1	22.5	19.395	15.4	16.5	13.8	10.3	10.3	10.3	10.3	34	53.1	9	11.4	1	1.6	1	1.6	
8	Nearly disease-free.	93	95.8	58	70.8	1	1.0	54.0	44.1	18.1	74.1	53.4	31.8	27.855	15.5	23.3	10.5	14.4	9.0	45	66.8	18	31.6	4	7.0	4	7.0	4	7.0	
9	Moderately diseased.	91	94.8	50	52.1	6	6.3	50.1	39.1	22.0	62.5	34.8	21.9	18.484	17.7	15.1	17.3	14.8	17.1	12.0	35	44.3	21	26.6	17	21.5	11	13.5		
10	Nearly disease-free.	91	94.8	50	52.1	6	6.3	50.1	39.1	22.0	62.5	34.8	21.9	18.484	17.7	15.1	17.3	14.8	17.1	12.0	35	44.3	21	26.6	17	21.5	11	13.5		
11	Moderately diseased.	85	88.5	47	49.0	5	5.2	51.2	41.4	18.1	69.7	46.8	25.8	21.864	15.6	17.9	14.6	12.3	14.3	10.5	37	59.6	14	19.2	14	19.2	14	19.2		
12	Nearly disease-free.	80	81.5	50	51.3	2	2.0	51.2	41.4	18.1	69.7	46.8	25.8	21.864	15.6	17.9	14.6	12.3	14.3	10.5	42	59.6	6	8.0	6	8.0	6	8.0		
13	Moderately diseased.	90	93.8	48	50.3	3	3.3	50.3	39.3	20.8	65.8	40.7	23.1	25.844	16.9	27.8	13.5	15.5	11.5	43	54.4	20	24.0	16	18.2	7	8.9			
14	Nearly disease-free.	88	91.6	49	49.9	10	10.4	43.2	34.2	21.4	77.2	47.2	33.5	33.9	20.6	17.3	14.6	12.3	14.3	10.5	44	53.4	23	27.9	12	15.7	7	8.3		
15	Moderately diseased.	82	85.7	47	49.2	6	6.3	50.3	41.7	22.8	68.8	39.7	18.5	15.844	16.6	23.2	13.2	13.2	13.2	13.2	13.2	43	54.4	20	24.0	12	15.0	7	8.3	
16	Nearly disease-free.	87	90.6	54	56.3	3	3.1	52.0	42.4	18.5	71.0	46.8	27.8	24.066	14.6	17.9	14.6	12.3	14.3	10.5	42	59.6	6	8.0	6	8.0	6	8.0		
17	Moderately diseased.	87	90.6	54	56.3	3	3.1	52.0	42.4	18.5	71.0	46.8	27.8	24.066	14.6	17.9	14.6	12.3	14.3	10.5	42	59.6	6	8.0	6	8.0	6	8.0		
18	Nearly disease-free.	91	94.8	50	52.1	6	6.3	50.1	39.1	22.0	62.5	34.8	21.9	18.484	17.7	15.1	17.3	14.8	17.1	12.0	35	44.3	21	26.6	17	21.5	11	13.5		
19	Moderately diseased.	87	90.6	54	56.3	3	3.1	52.0	42.4	18.5	71.0	46.8	27.8	24.066	14.6	17.9	14.6	12.3	14.3	10.5	42	59.6	6	8.0	6	8.0	6	8.0		
20	Nearly disease-free.	91	94.8	50	52.1	6	6.3	50.1	39.1	22.0	62.5	34.8	21.9	18.484	17.7	15.1	17.3	14.8	17.1	12.0	35	44.3	21	26.6	17	21.5	11	13.5		
21	Average of moderately diseased.	86.9	86.1	46.1	46.1	7.0	7.0	47.2	37.2	21.2	61.9	32.0	19.1	16.566	15.1	23.0	14.2	15.1	11.0	42.8	54.8	23.0	26.8	12.0	13.9	7.7	7.7	7.7	7.7	
22	Average of nearly disease-free.	94.2	94.2	57.3	57.3	4.1	4.1	53.2	42.3	18.7	70.9	46.9	29.4	24.941	16.3	26.8	13.9	11.7	11.3	9.9	47.6	57.6	21.6	26.8	12.0	13.9	7.7	7.7	7.7	7.7
23	Total average.	90.3	90.3	51.7	51.7	5.5	5.5	49.2	39.8	19.9	66.3	34.5	24.3	21.253	15.7	23.4	14.6	12.9	11.2	45.2	56.2	22.0	26.8	12.0	13.9	7.7	7.7	7.7	7.7	

of nearly diseased entirely diseased	0.7	56.8	14.5	50.0	35.0	29.2	28.3	9.1
Percentage decrease of nearly diseased from seed that moderately dis- eased seed.								

a 99.7 per cent viable and 69.8 per cent diseased.      b 100 per cent viable and 3.3 per cent diseased.



Percentage decrease in mean yield of mod- erately diseased	Percentage decrease in free seed yield under moderately dis- eased seed	Percentage decrease in mean yield of mod- erately diseased	Percentage decrease in free seed yield under moderately dis- eased seed
7.7	40.8	10.5	35.0
64.9			8.3



TABLE XXIV.—Comprehensive study of the principal data showing the influence of moderately diseased and nearly disease-free seed on early vigor, yield of marketable corn, and other related factors. Reid Yellow Dent grown on infested soil of medium fertility. Fourth planting (May 30) of the same series shown in Tables XXI, XXII, and XXIII. Bloomington, Ill., 1921

Soil plot No.	Condition of seed.	Early vigor data.					Yield data.												Sound nubbins.									
		Total stand.		Vigorous plants.		Weak plants.	Original weight.	Air-dry weight.	Shrinkage.	Total yield per acre.		Marketable corn.			Unmarketable corn.			Market-able ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Number.	Per cent.			
		Number.	Per cent.	Number.	Per cent.	Number.	Lbs.	Lbs.	P. ct.	Bu.	Bu.	Yield per acre.	Weight before shelling.	Weight shelled.	Shelling per-centage.	Moisture per-centage.	Weight of dry matter.	Weight before shelling.	Weight shelled.	Shelling per-centage.	Moisture per-centage.	Weight of dry matter.	Number.			Per cent.	Number.	Per cent.
1	Moderately diseased.	86	89.6	44	45.8	11	11.5	42.3	21.9	64.4	27.0	17.8	14.782	63.2	11.7	24.5	20.150	10.3	25	32.9	34	43.0	11	11.9	11	11.9	8	10.2
2	Nearly disease-free.	91	94.8	65	67.7	1	1.0	47.0	24.5	64.3	27.0	35.5	30.385	61.3	11.5	25.1	20.150	18.0	10	10	35.0	46.0	13	13.0	13	13.0	9	9.0
3	Moderately diseased.	86	91.8	78	81.3	15	15.0	47.0	24.5	64.3	27.0	41.9	34.682	62.0	11.5	25.1	20.150	18.0	10	10	35.0	46.0	13	13.0	13	13.0	9	9.0
4	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
5	Moderately diseased.	95	90.0	72	78.0	3	3.1	45.9	24.5	64.3	27.0	30.1	25.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
6	Nearly disease-free.	88	91.0	66	68.8	8	8.3	41.4	21.2	64.3	27.0	19.2	16.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
7	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
8	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
9	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
10	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
11	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
12	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
13	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
14	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
15	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
16	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
17	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
18	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
19	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
20	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
21	Moderately diseased.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
22	Nearly disease-free.	81	86.4	58	60.4	11	11.5	43.9	22.5	66.9	27.9	18.0	15.083	49.0	12.1	25.4	20.810	10.6	24	33.8	35	49.3	5	7.0	4	4.5	4	4.5
Average of moder-ately diseased.		88.3	91.0	64.0	64.0	9.1	9.1	44.0	24.5	66.0	30.6	19.3	16.283	49.3	12.1	25.4	20.418	10.7	30.5	39.5	38.2	43.0	10.7	13.0	10.7	13.0	5.1	5.1
Average of nearly disease-free.		91.3	94.8	75.0	75.0	3.3	3.3	50.5	29.0	70.4	34.9	31.9	29.118	51.7	12.1	25.4	20.417	10.6	61.1	61.1	61.1	61.1	23.6	23.6	23.6	23.6	4.6	4.6
Difference.		3.0	3.8	11.0	11.0	4.2	4.2	6.5	4.5	14.4	14.3	12.6	2.835	2.4	3.4	3.4	3.4	3.4	10.6	10.6	10.6	10.6	2.3	2.3	2.3	2.3	0.7	0.7

Percentage increase in yield of maize free of rot disease under moderately diseased seed.	a 99.7 per cent viable and 69.8 per cent diseased.										b 100 per cent viable and 3.3 per cent diseased.									
	3.4	17.2	64.8	15.1	79.4	67.4	49.3	14.0	23.8											
Percentage increase in yield of maize free of rot disease under moderately diseased seed.	3.4	17.2	64.8	15.1	79.4	67.4	49.3	14.0	23.8											

As the season advanced and conditions became more favorable for the growth of the weaker plants the difference in field stand became less, being only 3.0 per cent in the last planting (fig. 14).

TABLE XXV.—Summary of data in Tables XXI to XXIV inclusive, showing percentage increases in favor of nearly disease-free seed over moderately diseased seed.

Date of planting.	Percentage increase in favor of nearly disease-free seed.				
	Total stand.	Vigorous plants.	Total yield.	Yield of marketable corn.	Marketable ears.
May 7	15.0	53.4	17.2	49.3	31.6
14	9.7	56.8	14.5	50.0	35.0
21	7.7	49.8	10.5	35.8	33.0
30	3.4	17.2	15.1	79.4	67.4

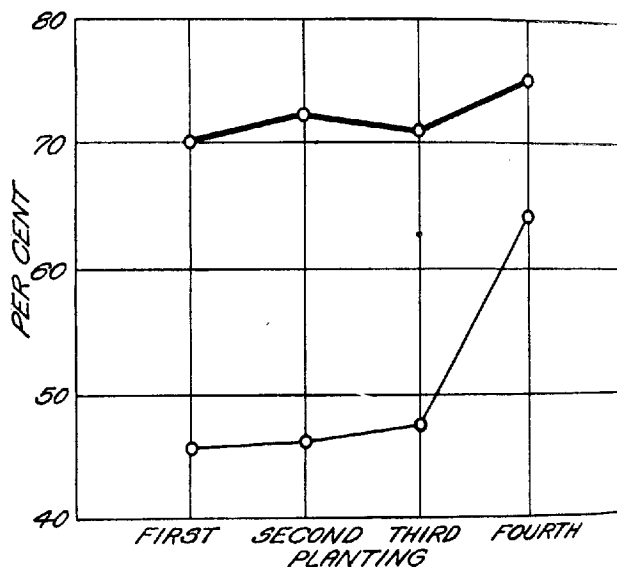


FIG. 15.—Graphic presentation of the percentage of kernels in the two lots of seed which produced vigorous plants when planted at different dates. The heavy line represents the nearly disease-free seed, the light line the moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

From all these data it will be seen that reduction in percentage of field stand, due to the use of infected or susceptible seed, depends on several factors, among which are the extent of infection and the general condition of the soil in which the seed is planted. There seems to be little doubt that the planting of seed infected with or susceptible to the root and stalk rot pathogens is responsible for many unsatisfactory

field stands and constitutes an important loss to the corn crop. This is especially true with corn planted early when weather and soil conditions are more or less unfavorable for germination and growth. However, a good field stand grown from diseased or disease-susceptible seed is no insurance against reduction in total yield. This fact is brought out in Table XXVI where the difference in field stand is only 3.0 per cent and the difference in total yield is 10.4 bushels per acre. It also occurs in Table XXIV where there is a difference of 3.4 per cent in field stand and 7.0 bushels in total yield. Moreover, in view of the results obtained by Kiesselbach and Ratcliff (25), it does not appear probable that the reductions in total field stand are an adequate explanation for all the reductions

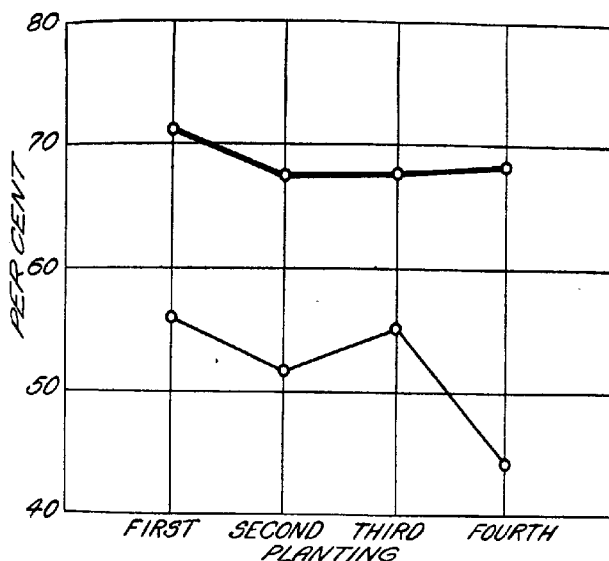


FIG. 16.—Graphic presentation of the percentage of marketable corn (by weight) produced by the two lots of seed at different dates of planting. The heavy line represents the nearly disease-free seed, the light line moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

in total yield in the experiments herein reported. An analysis of all the data shows that reduced stands may be considered, in a great majority of instances, an indication that a considerable portion of the corn population in question has grown from infected or susceptible seed which in turn is responsible for much of the reductions in total yield. The fact remains, however, that reductions in total yield herein reported can be traced to the use of infected seed and represent losses encountered in actual farm practice.

The reductions in early vigor in the corn grown from moderately diseased seed were well marked, with a very few exceptions, throughout all the experiments reported for 1921. The differences of  $24.4 \pm 2.14$ ,  $26.2 \pm 2.38$ ,  $23.6 \pm 2.46$  and  $11.0 \pm 2.21$  in percentage of vigorous plants

presented in Tables XXI to XXIV, and figures 9 to 12 and figure 15 are very significant. (Pl. 7.) Inasmuch as the majority of weak plants are either barren or nubbin producers, the differences in percentage of weak plants probably contributed to the wide differences in yield at the end of the season.

The differences of  $17.0 \pm 1.89$ ,  $16.0 \pm 1.74$ ,  $12.8 \pm 1.79$ , and  $24.3 \pm 2.59$  bushels per acre of sound corn are greater than the differences in total yield and are of greater economic importance to corn growers. This is

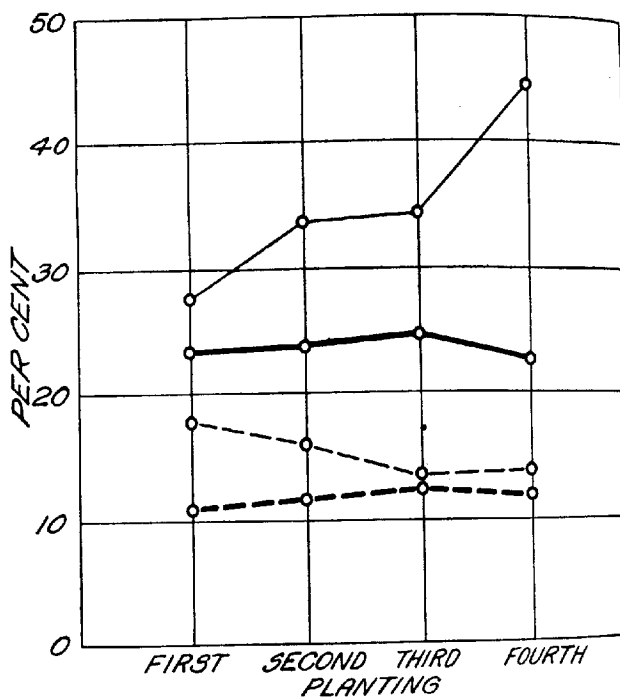


FIG. 17.—Graphic presentation of the total number of rotted ears (continuous lines) and the total number of chaffy ears (broken lines) produced by the two lots of seed at different dates of planting. The heavy lines represent the nearly disease-free seed, the light lines moderately diseased seed. (Taken from Tables XXI to XXIV, inclusive.)

especially true in seasons like 1921 when conditions were so favorable for the development of ear rots. The differences in the yield of marketable corn presented in Tables XXI, XXII, XXIII, and XXIV and figures 9, 10, and 11 are similar to those reported for 1920 and are well correlated with differences in early vigor (fig. 13). However, the results from the fourth planting that are presented in Table XXIV require some additional explanation. Although the difference in early vigor was the least in the last planting, yet the difference in yield of sound corn in this same planting was the greatest (Table XXV and fig. 12). The late-planted

corn suffered more from the combination of ear worm attack and weather conditions favorable to the development of ear rots. Both lots of corn were attacked approximately to the same extent by the earworms, but the corn grown from moderately diseased seed proved to be much more susceptible to the attacks of fungi following earworm injury than corn grown from nearly disease-free seed. Only 36.5 per cent of the ears from the moderately diseased seed were sound, while 61.1 per cent of the ears from the nearly disease-free seed were sound. Figure 16 presents graphically the percentage of marketable corn by weight produced by the moderately diseased and nearly disease-free seed from the different dates of planting.

A further analysis of the data, graphically presented in figure 17, shows that the increases in unmarketable corn in the later plantings from the moderately diseased seed were due to the increased number of rotted ears and not to any increase in number of chaffy ears. The decrease in the percentage of chaffy ears in the last three plantings from the moderately diseased seed is accounted for by the increased number of chaffy ears that were rotted. Ears and nubbins both rotted and chaffy were placed in the rotted class, since the rotting was considered more detrimental than the chaffiness. The decreased percentage of rotted ears in corn grown from the nearly disease-free seed as compared with that grown from the moderately diseased seed is very significant and indicates that the corn grown from nearly disease-free seed possessed considerably more resistance to injury by the fungi causing ear rots, as well as more resistance to the root and stalk rots, when planted in infested soil.

Additional data, similar to those in Tables XXI and XXII, are presented in Table XXVI. A comparison of the field performance of the corn planted from the three different seed lots, varying considerably in percentage of infection, shows that the diseased seed is associated with reduced field stand, reduced percentage of vigorous plants, increased percentage of weak plants, and reduced total yield, as well as reduced yield of sound, marketable corn. A considerable part of the increased yield of sound corn from the nearly disease-free seed is due to the smaller percentage of rotted ears.



Average of moderately diseased	88.3	70.2	4.4	50.7	11.0	0.5	71.9	50.0	31.1	38.1	27.1	25.8	17.0	14.1	12.7	50.9	60.8	24.4	3.2	11.6
	$\pm 1.14$	$\pm 2.33$	$\pm 0.71$	$\pm 0.77$	$\pm 1.0$	$\pm 0.5$	$\pm 0.59$	$\pm 1.17$	$\pm 0.6$	$\pm 1.1$	$\pm 1.2$	$\pm 1.1$	$\pm 1.0$	$\pm 1.1$	$\pm 1.2$	$\pm 1.1$	$\pm 1.70$	$\pm 1.77$	$\pm 0.41$	$\pm 1.15$
Average of slightly diseased	91.6	78.7	2.4	62.7	57.4	8.5	78.3	57.8	42.0	35.9	32.8	31.3	27.6	18.2	12.6	52.0	68.7	19.8	2.4	9.1
	$\pm 1.18$	$\pm 2.96$	$\pm 0.54$	$\pm 0.54$	$\pm 1.12$	$\pm 1.15$	$\pm 1.15$	$\pm 1.12$	$\pm 0.6$	$\pm 0.85$	$\pm 0.8$	$\pm 0.7$	$\pm 0.6$	$\pm 0.5$	$\pm 0.6$	$\pm 0.5$	$\pm 1.58$	$\pm 1.64$	$\pm 0.60$	$\pm 2.03$
Average of nearly disease-free	97.0	85.8	1.9	68.7	62.4	9.2	85.3	64.8	47.9	40.8	35.3	34.7	38.3	14.6	12.3	51.6	73.0	18.4	3.4	5.2
	$\pm 1.06$	$\pm 1.44$	$\pm 0.39$	$\pm 0.39$	$\pm 1.12$	$\pm 0.83$	$\pm 1.12$	$\pm 0.83$	$\pm 0.6$	$\pm 0.85$	$\pm 0.8$	$\pm 0.7$	$\pm 0.6$	$\pm 0.5$	$\pm 0.6$	$\pm 0.5$	$\pm 0.92$	$\pm 0.89$	$\pm 0.41$	$\pm 0.30$
Difference between moderately diseased and nearly disease-free																				
Percentage increase of nearly disease-free seed over moderately diseased seed	8.7	15.6	2.5				12.0	15.8									12.2	6.0		6.4
	$\pm 1.57$	$\pm 2.37$	$\pm 0.77$				$\pm 1.37$	$\pm 1.44$									$\pm 1.85$	$\pm 1.98$		$\pm 1.16$
Percentage decrease of nearly disease-free seed over moderately diseased seed	9.9	22.2					16.4	31.6									20.1			
			36.8															24.6		55.2



Some strains of Reid Yellow Dent have proved to be very susceptible to the root, stalk, and ear rot diseases when planted in infested soil or when the seed was inoculated at planting time with *Gibberella saubinetii*. Frequently ears of such strains of corn are so badly infected that it is impossible to select from them seed that is reasonably disease-free, regardless of the number of ears tested on the germinator. Seed from such a strain of Reid Yellow Dent was included in the inoculation experiments conducted in cooperation with Dr. James G. Dickson in 1921 on clean and infested soil, including early and late plantings. Corn grown from this seed proved to be very susceptible to injury by the organism (*Gibberella saubinetii*) with which it was inoculated. The corn in the inoculated plots had a slightly reduced stand and a much lower percentage of vigorous plants than in the uninoculated plots planted with the same lot of seed. The differences in percentages of strong plants in corn grown from this diseased, susceptible seed and in that grown from the nearly disease-free seed were very similar to those reported in Tables XXI to XXIV and XXVI. The very best seed that could be selected from this strain by use of the germination test averaged 27.5 per cent

TABLE XXVII.—Total yield and yield of marketable corn as influenced by diseased,<sup>a</sup> susceptible,<sup>b</sup> and nearly disease-free<sup>c</sup> seed planted at different dates on clean soil and infested soil at Bloomington, Ill., in 1921

INFESTED SOIL														
Date of planting.	Condition of seed.	Total stand.		Yield data.										
		Number.	Per cent.	Total yield per acre.	Marketable corn.			Unmarketable corn.			Rotted ears and rot- ted tubers.	Chaffy tubers.	Severely weakened.	Total tubers.
					Yield per acre.	Percentage by weight.	Test per bushel.	Yield per acre.	Percentage by weight.	Test per bushel.				
May 10	Original composite of susceptible seed <sup>a</sup> .....	312	86.7	77.6	48.0	61.8	54.5	29.6	38.2	48.7	53.9	34.6	2.7	9.1
	Selected composite of susceptible seed <sup>b</sup> .....	333	92.5	78.3	50.0	63.9	54.2	28.3	36.1	47.7	54.2	34.9	2.6	8.5
	Nearly disease-free composite <sup>c</sup> .....	354	98.3	89.7	69.3	77.2	58.5	20.4	22.8	52.5	71.3	17.7	3.2	7.3
	Original composite of susceptible seed.....	320	88.8	76.5	37.7	49.3	52.0	38.8	50.7	49.0	45.5	42.0	4.2	8.1
30	Selected composite of susceptible seed.....	334	92.8	71.8	39.7	55.3	52.1	44.7	49.2	49.5	49.5	2.4	7.3	
	Nearly disease-free composite.....	354	98.3	81.5	59.3	72.7	56.5	22.2	27.3	51.3	68.5	25.0	1.3	7.7
CLEAN SOIL														
11	Original composite of susceptible seed.....	319	88.6	81.0	51.8	63.9	53.5	29.2	36.1	49.2	54.7	30.8	6.9	7.6
	Selected composite of susceptible seed.....	336	93.4	75.7	42.5	56.1	54.5	33.2	43.9	48.7	48.0	32.5	8.8	10.7
	Nearly disease-free composite.....	346	96.1	90.6	73.0	80.5	58.3	17.6	19.5	50.8	71.1	19.0	1.9	8.1
	Original composite of susceptible seed.....	332	92.1	94.9	53.9	56.8	53.6	41.0	43.2	49.7	50.2	38.0	3.5	8.8
28	Selected composite of susceptible seed.....	345	95.8	95.4	55.1	57.8	53.8	40.2	42.2	50.0	51.5	37.0	4.0	7.7
	Nearly disease-free composite.....	353	98.2	104.7	81.6	77.9	57.8	23.1	22.1	52.6	73.9	16.2	1.6	8.6

<sup>a</sup> 96.5 per cent viable and 38.6 per cent diseased.

<sup>b</sup> 98.9 per cent viable and 27.5 per cent diseased.

<sup>c</sup> 99.9 per cent viable and 3.5 per cent diseased.

diseased. Although the complete data and discussion regarding these experiments are reserved for other publications, a limited number of these data are presented in Table XXVII to show the influence of seed infection and susceptibility to the root and stalk rots to total yield and yield of marketable corn, with special reference to the percentage of rotted ears.

The field stand data (Table XXVII) are of particular interest. Many investigators would consider a field stand of 92.5 to 95.8 per cent a satisfactory stand. These stand data lend further emphasis to the fact that satisfactory field stands are no guarantee against losses due to the root, stalk, and ear rot diseases.

TABLE XXVIII.—Summary of data presented in Table XXVII, showing percentages of rotted ears and yield of marketable corn

INFESTED SOIL				
Condition of seed.	Early planting.		Late planting.	
	Yield of marketable corn.	Rotted ears and nubbins.	Yield of marketable corn.	Rotted ears and nubbins.
	<i>Bushels.</i>	<i>Per cent.</i>	<i>Bushels.</i>	<i>Per cent.</i>
Selected composite of susceptible seed . . . . .	50.0	34.9	39.7	40.5
Nearly disease-free composite . . . . .	69.3	17.7	59.3	25.0
CLEAN SOIL				
Selected composite of susceptible seed . . . . .	42.5	32.5	55.2	37.0
Nearly disease-free composite . . . . .	73.0	19.0	81.6	16.0

Although there was a consistent reduction in total yield in the corn grown from the diseased, susceptible seed, the greatest and most important differences were in the yields of sound, marketable corn and the percentages of rotted ears. These items are summarized in Table XXVIII and figures 18 and 19. In both plantings and on both infected and clean soil the corn grown from diseased, susceptible seed produced more rotted ears and a much lower yield of marketable corn. The bushel weights indicate that the marketable corn produced by the nearly disease-free seed was of better quality than that produced by the diseased, susceptible seed.

The results secured at Urbana, reported in Table XXIX and figure 20, were obtained from corn grown from the same moderately diseased and nearly disease-free seed composites as the date-of-planting experiments conducted at Bloomington, reported in Tables XXI to XXV. The soil, however, was much higher in fertility than that of the Bloomington plots; in fact, the Urbana soil was in a better state of balanced fertility than the average Corn Belt soil will be for several years. The standards for classifying the plants into vigorous, semivigorous, and weak were based entirely on height measurements. Plants from the moderately diseased seed were grouped into three approximately equal divisions according to height. This same standard when applied to the plant populations from nearly disease-free seed gave relatively high percentages of vigorous plants and low percentages of weak plants.

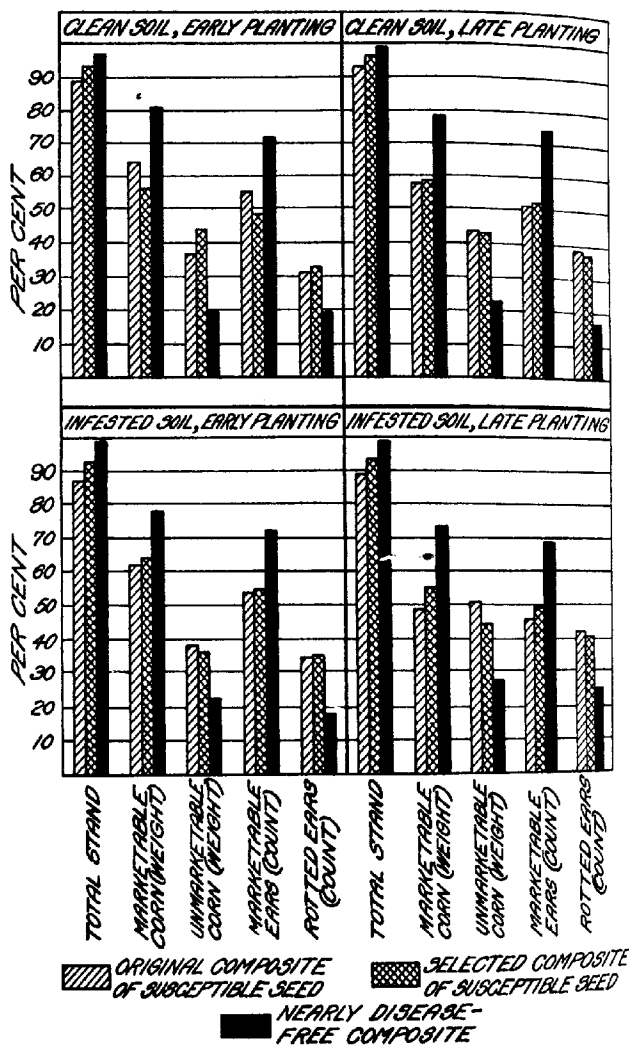


FIG. 18.—Graphic presentation of data presented in Table XXVIII.

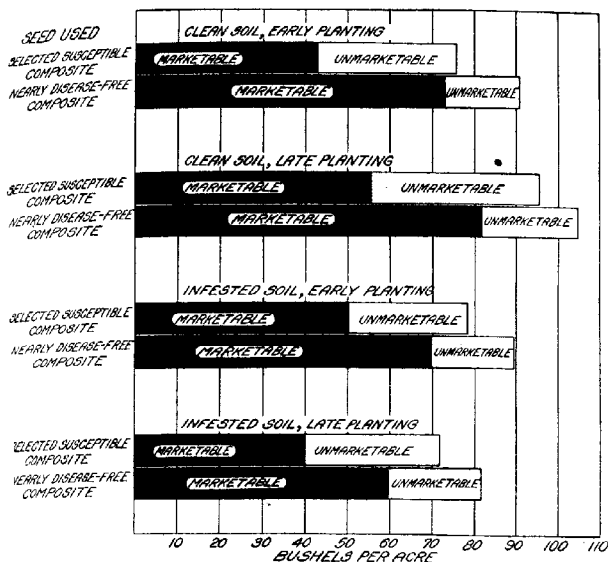


FIG. 19.—Graphic illustration of the total yield and yield of marketable and unmarketable corn (in bushels per acre) produced by a selected composite of susceptible seed and by nearly disease-free seed. Taken from Table XXVII.)

BLR XXIX.—Influence of an early date, two intermediate dates, and a late date of planting of moderately diseased and nearly disease-free seed on early vigor and yield. Reid Yellow Dent grown on infested soil of high fertility. Urbana, Ill., in 1921

No of planting	Condition of seed.	Early-vigor data.						Yield data.									
		Total stand.		Vigorous plants.		Weak plants.		Total yield per acre.	Marketable corn yield per acre.	Market-able ears.		Rotted ears and rotted nubbins.		Chaffy ears and chaffy nubbins.		Sound nubbins.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.			Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
1	Moderately diseased.....	1,464	86.8	352	21.6	681	39.1	Bus. 90.9	Bus. 73.4	902	67.2	174	13.0	130	9.7	130	10.1
	Nearly disease-free.....	1,599	95.2	914	58.1	123	6.4	100.7	89.6	1,110	76.1	114	7.8	80	5.5	154	10.6
10	Moderately diseased.....	1,448	85.7	385	24.9	513	28.2	86.3	69.4	790	64.3	170	13.9	128	10.4	140	11.4
	Nearly disease-free.....	1,626	96.4	1,362	81.1	88	5.0	99.8	85.2	1,022	72.3	138	9.8	128	9.0	126	8.9
20	Moderately diseased.....	1,477	88.7	578	34.2	410	21.5	77.0	57.4	746	58.1	190	14.8	170	13.2	178	13.9
	Nearly disease-free.....	1,593	94.8	1,028	61.5	211	13.5	90.5	79.1	1,004	73.2	128	9.3	96	7.0	144	10.5
31	Moderately diseased.....	1,691	88.2	496	29.8	452	23.9	79.0	55.9	860	61.4	192	13.7	122	15.9	126	9.0
	Nearly disease-free.....	1,784	92.9	1,199	65.5	55	2.9	89.9	76.6	1,144	72.9	166	10.6	114	7.3	144	9.2

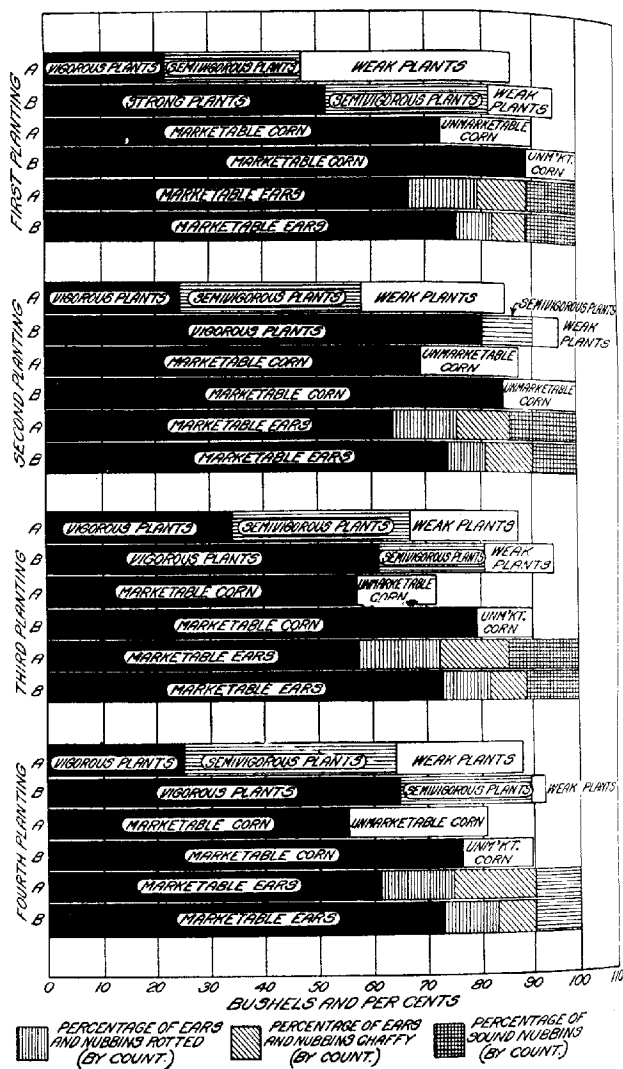


FIG. 20.—Graphic summary of Table XXIX, showing comparative results from planting moderate diseased (A) and nearly disease-free (B) seed on four different dates. Upper pair, vigor of seedlings (percentages); middle pair, relation of seedling vigor to acre yield in bushels of marketable and unmarketable shelled corn; lower pair, comparative percentages by number of marketable ears, rotted ears and nubbins, chaffy ears and nubbins, and sound nubbins.

## SUMMARY

1. Studies were conducted with diseased and apparently disease-free seedlings, transplanted from the germinator to the field. The disease-free seedlings made more rapid increases in height and circumference than the diseased seedlings during the first 25 days. The plants developed from the disease-free seedlings gave a much higher yield of grain than the plants from diseased seedlings.
2. Approximately 153,000 corn plants were classified during their early growth stages as vigorous, semivigorous, or weak. Of this number, more than 6,000 were harvested individually and the plant yields determined. The populations were grown from both diseased and relatively disease-free seed.
3. Early height and yield of corn plants were found to be correlated directly. In most cases the coefficient of correlation was sufficiently large to be significant.
4. Plants strong and vigorous in their early stages of growth produced a larger percentage of good-sized ears, and therefore higher yields of grain, and matured their grain somewhat earlier than the weaker ones, regardless of height at harvest time.
5. Plants weak in their early stages of growth usually produced nubbins only, or were barren.
6. Yields were recorded on the basis of marketable and unmarketable corn, the latter consisting of small nubbins, rotted ears, and light chaffy ears.
7. Nearly disease-free and moderately diseased seed were compared under different conditions in a number of experiments at Bloomington, Urbana, and elsewhere in Illinois.
8. Corn grown from seed not infected with the root and stalk rot pathogenes or not susceptible to the root and stalk rots had a higher percentage of strong, vigorous plants, a lower percentage of weak plants, and produced higher total yields and higher yields of marketable corn than corn grown under comparable conditions from infected or susceptible seed.
9. Corn populations grown from seed infected with one or more of the root and stalk rot pathogenes or susceptible to the root and stalk rots produced a much lower yield of sound, marketable corn and a larger percentage of nubbins, rotted ears, and light chaffy ears than corn grown from seed relatively free from and resistant to infections.
10. Corn earworms seem to show little preference between corn from diseased or nearly disease-free seed within a variety, but the total injury on the latter is much less because the worm injury is not followed by ear rots to the extent it is on the former.
11. Relatively disease-free seed ears selected from badly rotted plants proved to be inferior to relatively disease-free seed ears selected from apparently disease-free plants, especially when the comparisons were made on infested soil.
12. Not all lots of seed corn are suitable for selecting disease-free seed corn. Some types of corn are so susceptible to disease that seed which passes a good test on the germinator proves very susceptible when planted in infested soil.
13. Corn resistant to root and stalk rots is also resistant to ear rots.

## CONCLUSIONS

1. Early vigor and yield of the corn plant are closely correlated.
2. Variations in early vigor are due, not simply to chance, but to certain factors, genetic, physiologic, and pathologic, which are more or less under the control of the investigator, the corn breeder, and the corn grower.
3. The importance of the disease factor demands that it be given a prominent place in any program which aims at the permanent improvement of the corn crop.

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PLATE 1

Characteristic appearance of vigorous and weak young corn plants.

A.—A healthy, vigorous plant, grown from disease-free seed.

B.—A weak plant, stunted in size, and somewhat chlorotic, grown from diseased seed.

C.—A weak plant, chlorotic and partly blighted, grown from diseased seed.

D.—A weak plant, very chlorotic and nearly dead, grown from diseased seed.

All plants were of the same age and were drawn to the same scale.

PLATE 2

Representative ears of unmarketable corn.

A.—Rotted ears. This classification includes not only totally rotted ears but also those that are partly rotted. Ears of the latter kind may be composed largely of good kernels, but when shelled the rotted portion would greatly reduce the market grade of the whole.

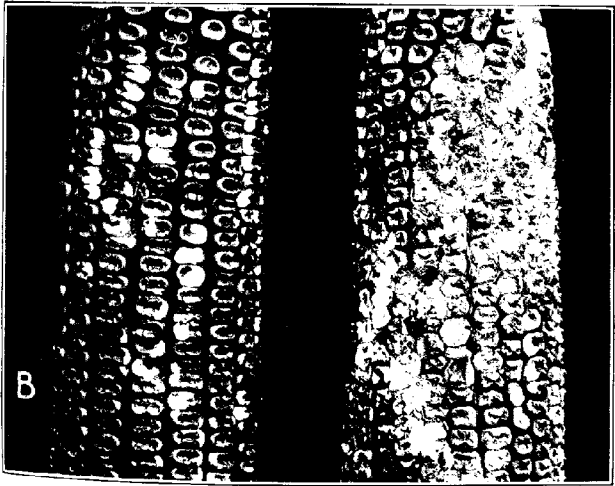
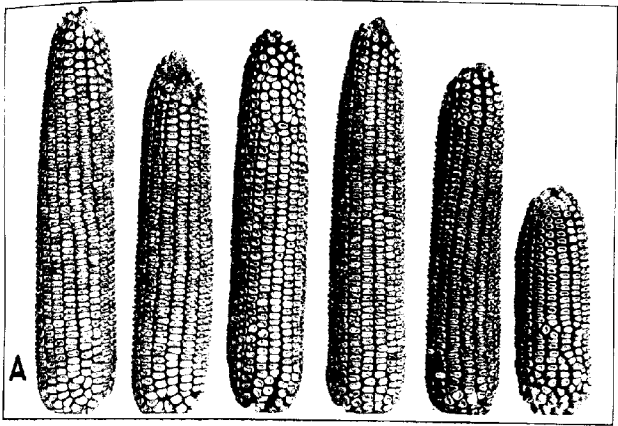
B.—Chaffy ears. There is some space between the kernels so that the ears can easily be bent and twisted. Such kernels are light and poorly developed and consequently have little market value. Chaffy ears that also showed signs of rot were placed in the rotted class shown in A because the rotting is more detrimental.

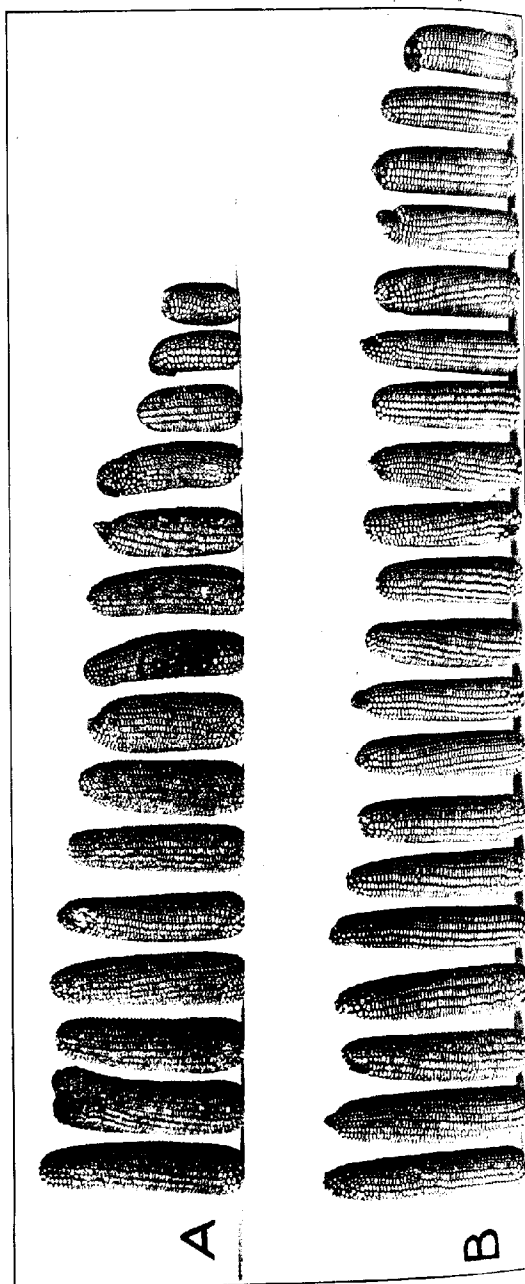
C.—Nubbins. Although most of the kernels here shown would be good for feeding purposes, most of these nubbins would be missed by the average corn husker. Furthermore, a large percentage of nubbins is produced by weak diseased plants.

PLATE 3

A.—Representative ears of marketable corn. Compare with unmarketable corn, Plate 2.

B.—On the left, an ear of a resistant type grown from disease-free seed and resistant to ear rots. On the right, an ear grown from diseased seed and susceptible to ear rots. Both ears were injured by the ear worm approximately to the same extent. On the resistant type (left) the injury was confined to that done by the worm, which injury was practically negligible as far as marketing is concerned. On the other ear (right, susceptible to ear rots, the worm injury was followed by fungous rots which made it practically worthless.





#### PLATE 4

Total yield from moderately diseased and disease-free seed of Reid Yellow Dent planted simultaneously in two parallel, 10-hill plots, at Bloomington, Ill.

A.—Product from 10 hills grown from moderately diseased seed. Ears seventh and eighth (from left) were chaffy, ninth and twelfth were partly rotted, and fifteenth was a rubbin.

B.—Product from 10 hills grown from disease-free seed. All these ears would be considered marketable.

PLATE 5

A.—Two hills of corn of same age, photographed 44 days after planting. The hill on the left contains two plants from diseased seed, the hill on the right three plants from disease-free seed.

B.—Same hills as in A, photographed 120 days after planting. The hill on the left produced 380 gm. and the hill on the right 930 gm. of air-dry shelled corn.







#### PLATE 6

Adjacent plots of Reid Yellow Dent planted at the same time, on uniform soil, and photographed on the same day, at Urbana, Ill.

A.—Plot grown from moderately diseased seed. This plot contained a large percentage of weak plants.

B.—Plot grown from nearly disease-free seed. This plot had 55 per cent more vigorous plants than the plot shown in A.

#### PLATE 7

Adjacent plots of Reid Yellow Dent planted at the same time, on uniform soil with rows the same distance apart and same number of kernels per hill, and photographed on the same day, at Bloomington, Ill.

A.—View down the center of four rows planted with moderately diseased corn.

B.—View down the center of adjacent four rows planted with nearly disease-free corn.

Note the increase in size and vigor of the plants in B. Note that in A leaves barely meet between the rows in the center of the picture while in B they overlap considerably.





## EFFECT OF BURNING ON VEGETATION IN KANSAS PASTURES<sup>1</sup>

By R. L. HENSEL<sup>2</sup>

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Burning pastures in the spring is a common practice on many Kansas farms. Those who practice burning contend that it reduces weeds, provides green feed at an earlier date and insures a better distribution of stock and more uniform grazing. It is also contended that no injury to the grass results. On the other hand, there are those who oppose burning on the ground that it does not cause growth to start earlier and that it injures the pastures by encouraging the growth of weeds and by decreasing the stand of desirable grasses. They admit that burned areas appear green earlier but maintain that the difference is apparent rather than real, for if a close examination is made it will be found that there are as many green plants on the unburned areas as on the burned. The accumulated surface litter covers the green plants in the former case.

No doubt the practice of grass burning is a relic of Indian days when it was customary for the different tribes to burn off certain, well-chosen areas in the spring. The main object apparently was to obtain fresh, green feed early in the year. Moreover, the areas made greener by burning would more likely attract game animals and hunting would be made easier.

This practice was perhaps passed on to the earlier settlers and has been continued to the present day. The objects now, however, are different. Today, one of the principal objects of burning pastures is to provide inexpensive forage for live stock early in the season, thereby eliminating the cost of the more expensive feed. In addition to this, there are always areas in the larger pastures which for various reasons are not fully grazed. The grass in these places grows rank and becomes unpalatable. Burning destroys this dead, unpalatable grass and permits the new growth to be grazed to better advantage. No experimental evidence relating to the effect of burning appears to be available, although it is clear that such information is needed in view of the importance of the question and the controversial opinions regarding it.

In order to secure such information, the Kansas Agricultural Experiment Station began a series of studies in 1918, which were designed to secure data on the question. These experiments were conducted about 9 miles north of Manhattan on a 1,500-acre tract of pasture land.<sup>3</sup> The

<sup>1</sup> Accepted for publication Aug. 18, 1922. Contribution No. 142 from the Department of Agronomy, Kansas Agricultural Experiment Station.

<sup>2</sup> The experiments discussed in this paper were inaugurated in 1918 by Mr. R. K. Bonnett. In 1919, Mr. C. W. Mullen had charge of the work until July 15. Since that time the work has been in charge of the author.

<sup>3</sup> Mr. Dan D. Casement of Manhattan cooperates with the Agricultural Experiment Station in the study of certain pasture problems on this tract of land.

land is typical eastern Kansas prairie. Using Clements' classification, the vegetation belongs in the "True Prairie" (*Stipa-Koeleria*) association.

#### TOPOGRAPHY AND SOIL

The topography is rolling and the land is too hilly for cultivation. The soil ranges from a dark brown to black loam with an underlying subsoil composed of clays and shales. There are many outcroppings of limestone, and many of the slopes and ridges are covered with loose chert rock. The elevation varies from 1,200 to 1,400 feet above sea level.

#### CLIMATIC CONDITIONS

The average, annual precipitation based on a 59-year period preceding 1918 is 31.50 inches. Table I shows the monthly precipitation at Manhattan for the duration of these experiments—namely, from 1918 to 1921 inclusive. It will be noticed that the rainfall during each of these years was less than the 59-year average, indicating that conditions, so far as rainfall was concerned, were subnormal but probably insufficient to influence the experiments to any marked degree.

The average date of the last killing frost in the spring at Manhattan is April 25 and that of the first killing frost in the fall is October 8, both based on the 59-year period. The average frost-free period is, therefore, 166 days. The growing season for the principal native grasses is much longer, however.

TABLE I.—Precipitation by months at Manhattan, Kans., from 1918 to 1921 and the 59-year average

Month.	Precipitation.				
	1918.	1919.	1920.	1921.	1859 to 1917: (average).
	Inches.	Inches.	Inches.	Inches.	Inches.
Jan.....	0.65	0.26	0.13	1.08	0.81
Feb.....	.52	1.24	.55	.36	1.20
Mar.....	.76	5.03	.20	.16	1.40
Apr.....	4.60	3.40	4.09	2.95	2.90
May.....	5.28	3.15	1.75	3.32	4.02
June.....	1.56	4.66	2.24	6.26	4.57
July.....	1.98	1.45	4.83	4.21	4.07
Aug.....	3.34	1.43	6.76	4.65	3.66
Sept.....	2.35	2.65	4.39	3.17	3.24
Oct.....	4.87	.68	.90	1.38	2.20
Nov.....	1.55	2.62	2.20	0.00	1.57
Dec.....	2.30	.08	1.35	0.00	.97
Total.....	29.76	27.65	28.39	27.54	31.50

The principal growth takes place from March 15 to July 1. If rainfall is deficient in March, April, and May a "backward" season results, but this may be made up by heavy rains in July and August, which will start vigorous growth, resulting in abundant late summer pasture.

<sup>1</sup> CLEMENTS, Frederic E. PLANT INDICATORS. XVI, 388 p., 25 fig., 92 pl. Washington, D. C. 1922. Bibliography, p. 364-373. (Carnegie Inst. Wash. Pub. 290.)

## VEGETATIVE COVER

The principal grasses are big bluestem (*Andropogon furcatus* Muhl.), little bluestem (*Andropogon scoparius* Michx.), side oats grama (*Bouteloua curtipendula* (Michx.) Torr.), and Kentucky bluegrass (*Poa pratensis* L.). Grasses of lesser importance are blue grama (*Bouteloua gracilis* H. B. K.), hairy grama (*Bouteloua hirsuta* Lag.), sand drop seed (*Sporobolus cryptandrus* (Torr.) Gray), switch grass (*Panicum virgatum* L.), and Indian grass (*Sorghastrum nutans* (L.) Nash). Common weeds are annual ragweed (*Ambrosia artemisiifolia* L.), perennial ragweed (*Ambrosia psilostachya* D. C.), prairie sage (*Artemisia gnaphaloides* Nutt.), prairie ragwort (*Senecio platensis* Nutt.), dwarf fleabane (*Erigeron divaricatus* Michx.), snow-on-the-mountain (*Euphorbia marginata* Pursh.), and others of lesser importance. There is considerable wolfberry (*Symphoricarpos occidentalis* Hook.) on some of the overgrazed pastures. Two sedges, *Carex pennsylvanica* Lamarck and *Carex meadii* Dewey, are common over the entire region.

## EXPERIMENTAL METHODS

The experiments were planned to obtain data on the following points:

1. The effect of burning on the temperature of the soil during the early growing season (March 15 to June 10) at depths of 1 and 3 inches below the surface.
2. The effect of burning on grasses.
3. The effect of burning on weeds.
4. The effect of burning on the yield.

The experiments were conducted over an area 75 by 150 feet on a ridge top with a slight southern slope. The area was divided into two sections. One of these, called section A, was burned as early each year as conditions would permit and the other, section B, was never burned.

Section A (Pl. 1, A) was burned March 23, 1918, March 13, 1919, April 2, 1920, and April 7, 1921, the variations in dates being due to seasonal conditions. The burning was usually conducted shortly after a rain in order to avoid possible injury to the roots and also to afford better control of the fire.

The number of plants of each species of grasses, sedges, and weeds, the temperature of the soil, the time at which growth began in the spring each year, and the yield of forage was determined for each area. The yields were generally determined in September of each year. The accumulated growth was cut and removed from the plots (Pl. 1, B) immediately after. It was planned to leave as much material as is usually left by livestock in order to allow the plots to remain in a condition comparable to a grazed area, although it might have been better to have turned in a few head of livestock. However, this would have disturbed the permanently staked quadrats and probably would have interfered with the main purpose of the experiments.

It was originally planned to take soil samples on each plot at regular intervals, but this could not be done because of the rocky soil of the area.

## DETERMINING THE TEMPERATURE

The four instruments used for obtaining soil temperatures were Columbia recording thermographs fitted with soil bulbs. Two were on the plot which was burned each year; one recorded temperatures at 1



inch under the surface and the other at 3 inches. Two others on the plot which was never burned also recorded the temperature at depths of 1 and 3 inches. The thermographs were kept in operation continuously from the time the field work began early in the spring until the latter part of June or the first of July.

#### QUADRAT CHARTINGS

In order to study the effect which the different treatments had on the principal plants, 7 small quadrats, each 10 inches square, were laid out in each plot. (Pl. 2, A.) The quadrats were bounded by a wooden frame, across which, in both directions, were woven strands of ordinary fine twine, held in place by small nails driven into the frame. These were placed at intervals of one-half inch. The twine divided each quadrat into 400 squares, each one-half inch on a side. These frames were permanently staked down so that exactly the same area would be charted each time. Sheets of paper similarly divided were used for recording the data, and upon these the observer placed the exact composition as he found it in each square of the quadrat. On account of the small size of the squares, it was not necessary to sketch the size of each grass tuft (Pl. 2, B). Instead, the results were compiled by squares for grasses and sedges and by specimens for the weeds. For example, quadrat A, 1 might contain 150 squares of big bluestem, 75 of little bluestem, and 15 specimens of dwarf fleabane. The method of using the squares as the basis of measurement, rather than the exact area occupied by each plant is not generally recommended, but in this case it was used, not only because it simplified the work but also because of the small size of both the individual grass plants and the squares on the quadrat frame. It has proved successful and has shown the relative change from year to year in a satisfactory way.

#### YIELDS

In order to arrive at the difference in amount of forage actually produced on the different plots, a number of cuttings were made on each area. This was generally done in September. An iron hoop, the rim of which included an area of one ten-thousandth of an acre, was used. The hoop was permitted to fall more or less at random and the material within the borders cut 1 inch above ground with a pair of sheep shears. The material was then dried and weighed and the yields per acre calculated.

#### EXPERIMENTAL RESULTS

##### THE EFFECT OF BURNING ON GRASSES

Quadrats on both the early-burned area and the unburned area were charted three times each season. From this data the number of squares which contained grasses, sedges, and weeds was compiled. A summary of the data on grasses and sedges is given in Table II. The dates of burning were March 23, 1918, March 13, 1919, April 2, 1920, and April 7, 1921.

TABLE II.—*Number of squares on all quadrats containing grasses and sedges (1918 to 1921)*

Date of charting.	Burned area.			Unburned area.		
	Grasses.	Sedges.	Both.	Grasses.	Sedges.	Both.
First:	<i>Squares.</i>	<i>Squares.</i>	<i>Squares.</i>	<i>Squares.</i>	<i>Squares.</i>	<i>Squares.</i>
Apr. 9, 1918.....	234	87	321	21	34	55
Apr. 17, 1919.....	308	114	422	163	75	238
May 5, 1920.....	621	39	660	492	25	517
May 3, 1921.....	665	40	705	548	64	612
Average.....	457	70	527	306	49	355
Second:						
Apr. 30, 1918.....	437	120	557	131	66	197
May 9, 1919.....	746	213	959	572	86	658
May 22, 1920.....	761	45	806	697	116	813
May 27, 1921.....	714	51	765	560	93	653
Average.....	665	107	772	490	90	580
Third:						
June 3, 1918.....	538	145	683	486	73	559
May 31, 1919.....	758	172	930	601	118	719
June 20, 1920.....	702	105	807	660	149	809
July 1, 1921.....	652	115	767	524	145	669
Average.....	662	134	796	568	121	689

The most striking and perhaps the most significant differences appear in the first charting. It will be noticed that there is almost a month's difference between the earliest first charting date and the latest first charting.

In every season there was a\*much larger number of squares containing grasses on the burned than on the unburned areas. In 1918 there were ten times as many squares containing grasses on the burned area as on the unburned; in 1919, the difference was 88 per cent; in 1920, 26 per cent; and in 1921, 21 per cent. The decrease in the difference is no doubt due in part at least to the later charting dates in 1919, 1920, and 1921. Therefore the essential and important conclusion is that in each year there were more grasses on the burned area. This substantiates the opinion of those advocates of burning who claim that it makes green feed available at an earlier date.

Burning seems to have decreased the number of sedges while no consistent change in this respect can be observed on the unburned areas. Just why burning injured the sedges more than the grasses is not clear.

The second charting of each year showed a marked increase in the number of specimens as compared with the first charting. This is to be expected and is due to the additional growth which takes place as the season advances. The paramount factor, however, is the greater number of grasses for the burned area compared with the unburned. The increase of grass on the burned area was 233 per cent in 1918, 30 per cent in 1919, 9 per cent in 1920, and 30 per cent in 1921. The large difference in 1918 is due to the early charting date and substantiates the conclusion that growth began much earlier on the burned plot.

The number of sedges appears to have been decidedly increased by burning for two years while in the two other years a decrease was recorded.

When the third chartings were made most of the vegetative growth for the season had taken place. Nevertheless, it will be seen that in each year there were more squares containing grasses on the burned than on the unburned areas. The variation from year to year is no more than would normally be expected, since it is a well known fact that the amount of vegetation produced in any area may vary from 10 to 40 per cent. The variations shown in these tables are probably due, therefore, to seasonal conditions rather than to treatment.

Burning seems to have resulted in a decrease in the number of sedges, as they are decidedly fewer in the last two years of the experiment than in the first and second years. The number has increased somewhat on the unburned section. Since the number to begin with was less on the unburned areas, the effect of burning on these plants is not entirely clear. In this pasture, sedges are not common enough to be an important factor and hence no great importance can be attached to the changes so far recorded.

In order to give an idea of the changes in composition that are taking place on the two plots, Table III, which shows the species of grasses and sedges and their abundance in 1918 and in 1921, has been prepared.

TABLE III.—Average composition of all quadrats on burned and unburned areas in 1918 and in 1921 (third charting)

Species.	Burned area.		Unburned area.	
	June 3, 1918.	July 1, 1921.	June 3, 1918.	July 1, 1921.
	Squares.	Squares.	Squares.	Squares.
Big bluestem.....	165	138	133	232
Little bluestem.....	265	394	308	122
Side oats grama.....	88	12	40	11
Kentucky bluegrass.....	15	42	0	135
Others <sup>1</sup> .....	5	66	5	24
Sedges <sup>2</sup> .....	145	115	73	145
Total grasses.....	538	652	486	524

<sup>1</sup> Principally *Sporobolus heterolepis* Gray.

<sup>2</sup> Principally *Carex pennsylvanica* Lam. and *Carex meadii* Dewey.

The most marked effect of burning has been to decrease the amount of big bluestem and increase the amount of little bluestem. The decrease in big bluestem is especially noteworthy in view of its tendency to increase on the unburned plot. This change is very noticeable when the plots are examined. There has been an increase of 48 per cent in little bluestem on the burned plot and a decrease of 60 per cent on the unburned plot. Side oats grama decreased in both cases but the decrease is much greater on the unburned plots. No Kentucky bluegrass was recorded on the unburned plots in 1918 but in 1921 there were 135 squares containing this grass. On the burned plot there were 15 squares in 1918, but only 42 in 1921, a very small increase as compared with the unburned plots. Chief among the grasses listed as "others" is *Sporobolus heterolepis*. The amount of this grass present in the burned area is quite noticeable and it appears to be increasing.

It is probable that those grasses which start earliest in the spring are injured most. The injury by burning no doubt kills some plants and

weakens others so that adverse conditions later in the season may cause their death.

It would seem from the above that the effects of burning will depend somewhat upon the composition of the pasture. A big bluestem pasture, or a Kentucky bluegrass pasture, would apparently be injured unless these grasses are replaced by equally or more desirable grasses. In many cases this, no doubt, is what would happen. There are undoubtedly other instances where they would be replaced by much less desirable species, in which instance burning would result in injury.

#### EFFECT OF BURNING ON WEEDS

The number and kind of weeds present was also recorded at each charting. This compilation, however, was by specimens and not by squares, as was done with grasses. The principal object was to determine whether burning retards or increases the spread of weeds. If there is a steady increase, it can be assumed that retrogression is taking place, while if no increases occur it may be assumed that the grasses are holding their own. It should be noted that there is always a considerable variation in the number of weeds present on any given area from year to year. Weeds react very quickly to favorable or unfavorable growing conditions and this should be kept in mind when analyzing the data.

The principal weeds found in the order of their importance are prairie sage (*Artemisia gnaphaloides* Nutt.), dwarf horseweed (*Erigeron divaricatus* Michx.), annual ragweed (*Ambrosia artemisiifolia* L.), whorled milkweed (*Asclepias verticillata* L.), perennial ragweed (*Ambrosia psilostachya* D. C.), daisy fleabane (*Erigeron ramosus* Watt.), and prairie cat's foot (*Antennaria campestris* Rydberg).

Table IV shows the total number of weeds of all kinds present in all of the quadrats on the unburned and burned areas for each year.

TABLE IV.—Number of weed specimens present on the burned and unburned areas from 1918 to 1921

First charting.			Second charting.			Third charting.		
Date.	Burned.	Un-burned.	Date.	Burned.	Un-burned.	Date.	Burned.	Un-burned.
Apr. 9, 1918	None.	None.	Apr. 30, 1918	None.	None.	June 3, 1918	5	1
Apr. 17, 1919	55	23	May 9, 1919	85	53	May 31, 1919	80	92
May 5, 1920	45	38	May 22, 1920	47	60	June 20, 1920	37	41
May 31, 1921	31	48	May 27, 1921	41	61	July 1, 1921	29	50

When the first and second chartings were made in 1918 no weeds were found on either the burned or unburned areas. The third charting showed five specimens on the burned area and one on the unburned. These numbers are so small that but little importance can be attached to them except to say that the plots were almost free of weeds.

In 1919, there was a decided increase in the number of weeds. When the first charting was made, there were 55 specimens of weeds on the burned and 23 specimens on the unburned area. On the second date, there were 85 and 53 specimens, respectively, and on the third date 80 and 92, respectively.

The larger number of weeds on the unburned area in the third period may have been due to the later start of the vegetation on these plots. It may be concluded from these data that the treatment of the plots had nothing to do with the increase over 1918. If the burning was responsible for these changes, there would have been a greater difference in the number of weeds on the burned and unburned areas.

In 1920, weeds were generally less abundant than in the preceding year and there were no consistent differences due to the treatment of the plots. The 1921 burning reduced the number of weeds somewhat, the differences in each case apparently being significant.

The data seem to show beyond doubt that burning does not favor the growth of weeds but that on the contrary there is a tendency towards a decrease in their number on the burned plots as compared with the unburned plots.

#### THE EFFECT OF BURNING ON SOIL TEMPERATURES

The principal object of recording the soil temperature was to determine if burning resulted in a warmer soil early in the spring, and if so, whether this difference could be correlated with the growth of vegetation. The thermograph bulbs were placed at depths of one and three inches respectively, because the majority of roots are found at about the latter depth and because germinating seed is ordinarily found at a depth of one inch or less.

The thermographs were usually started immediately after burning and they were all kept running until June 10. In 1921, they were started before burning in order to see if the heat generated by the burning vegetation penetrated the soil. The records show that no rise in temperatures took place in either the one-inch or three-inch bulbs, indicating that the heat of combustion is confined entirely to the immediate ground surface. It seems, therefore, that unless there is considerable accumulation of vegetation the fire does not heat the soil sufficiently to cause material damage. Usually most plants are merely scorched and it is probable that weeds and grass seeds which are lightly covered escape injury entirely. Moreover if the burning is done soon after a rain, as was done in these experiments, the moisture in the ground will aid in protecting the seeds.

The maximum and minimum temperatures were assumed to be the most significant temperatures in relation to vegetation and are the only ones considered in this paper. The mean maximum and mean minimum for each year are presented in Table V which follows. Figures 5, 6, 7 and 8 have been prepared to show the change in temperature with the advance of the season for each year.

TABLE V.—Mean maximum and minimum temperatures at a depth of one inch on burned and unburned plots (1918 to 1921)

Year.	Mean maximum temperatures.			Mean minimum temperatures.		
	Burned.	Unburned.	Difference.	Burned.	Unburned.	Difference.
1918.....	81.7	67.2	+14.5	50.2	50.0	-0.4
1919.....	71.6	67.1	+4.5	54.7	51.9	+2.8
1920.....	88.9	70.3	+18.6	60.7	53.3	+7.4
1921.....	77.0	66.4	+10.6	49.6	42.8	+6.8
Average.....	79.8	67.7	+12.1	53.8	49.7	+4.1

TABLE VI.—Mean maximum and minimum temperatures at a depth of three inches on burned and unburned plots (1918 to 1921)

Year.	Mean maximum temperatures.			Mean minimum temperatures.		
	Burned.	Unburned.	Difference.	Burned.	Unburned.	Difference.
1918.....	69.9	61.0	+8.9	53.4	50.4	+3.0
1919.....	65.8	62.1	+3.7	54.7	51.9	+2.8
1920.....	72.6	72.8	-.2	61.8	55.5	+6.3
1921.....	66.6	64.8	+1.8	52.1	47.3	+4.8
Average.....	68.7	65.1	+3.6	55.5	51.3	+4.2

In every year, as shown in Table V, the mean maximum temperature of the soil at a depth of one inch, averaged materially higher on the burned plot. This difference ranged from 4.5° F. in 1919, to 18.6° in 1920. The average difference was 12.1°. A higher mean minimum temperature was also recorded for the burned plots except in 1918, when the difference was negligible. The average difference it will be noted is 4.1°.

Similar, although less marked differences were recorded at a depth of three inches (Table VI), where the average difference in the mean maximum temperature was 3.6° F. and in the mean minimum temperature 4.2°. These results show beyond a reasonable doubt that the soil on the burned plots absorbed more heat, which no doubt explains the earlier growth of vegetation previously pointed out.

As shown in figures 1, 2, 3, and 4, the burned plots were consistently warmer throughout the season than the unburned plots, as measured by the weekly mean maximum temperatures at a depth of one inch, with a few exceptions. The mean minimum temperatures were also in favor of the burned plots. It appears that the differences in early growth of vegetation may easily be accounted for by these differences in temperature.

#### EFFECT OF BURNING ON THE YIELD OF HAY

The yield of hay was usually determined about September 10, each year, by harvesting several areas, usually ten in number. These areas, each one-tenth of an acre, were selected by means of the iron hoop previously mentioned.

The results expressed in pounds of dry matter per acre are shown for each year in Table IX.

TABLE IX.—Showing the yields of cured hay on burned and unburned plots (1918 to 1921)

Year.	Pounds per acre.		
	Burned.	Unburned.	Difference (in favor of burned).
1918.....			
1919.....	3,075	4,623	-1,548
1920.....	3,500	3,633	-133
1921.....	1,730	1,774	-44
Average.....	3,156	2,973	+183
	2,865	3,251	-386

The large difference in the yield between the burned and unburned areas in 1918 is difficult to explain, since the quadrat chartings up until June 3 indicated considerably more shoots on the burned areas than on the unburned. This apparently should mean higher yields. It seems probable that in obtaining the cuttings a portion of the dried material remaining from 1917 was included.

There was a slight difference in yield in 1919 and in 1920, in favor of the unburned plots, but it is too small to be considered of much impor-

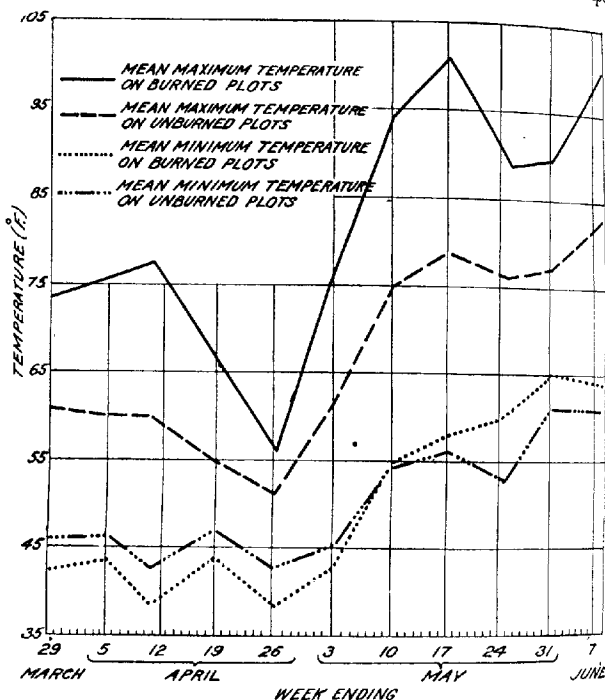


FIG. 1.—Graph showing weekly mean maximum and minimum temperatures in 1918 at a depth of 1 inch under the surface on burned and unburned plots.

tance. Conditions were unfavorable in 1920, and the grasses showed a poor growth.

In 1921 the burned area produced the higher yield, the difference being 183 pounds per acre. Conditions were subnormal in the early season. There was not much rainfall early in the spring and cold weather persisted until June. Later on, however, good rains fell and grass showed excellent growth. The average difference of 386 pounds per acre in favor of the unburned is largely due to the 1918 results. Considering the data for 1919, 1920, and 1921 only, it will be seen that the amounts of hay produced on the two areas are practically equal.

The general opinion among those who are opposed to burning is that much more forage is produced on areas not burned. It is believed that this conclusion is erroneous, since the experiments here reported have

ailed to show, so far, that much higher yields are actually produced on unburned areas. Considering the small difference in yield and the marked difference in the number of desirable grasses in favor of the burned plot, can not be concluded that burning has caused any injury to the pasture. The last word regarding burning has yet to be written. The results secured, as these experiments show, will likely depend not only on seasonal conditions but also upon the kind of grasses and other vegetation present, and more extensive experiments with a wide variety of conditions will be necessary to determine just what may in general be expected of burning.

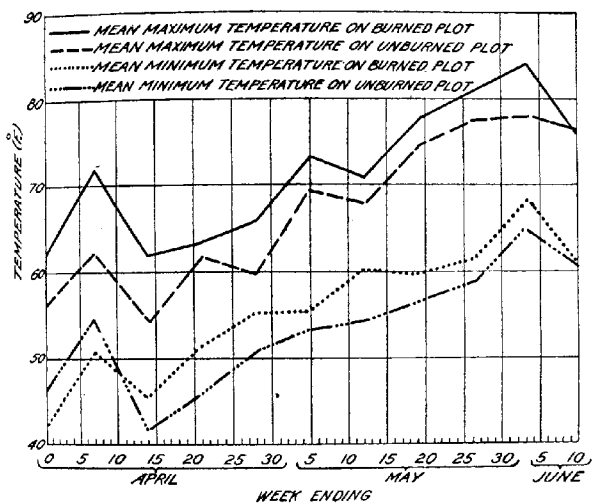


FIG. 2.—Graph showing weekly mean maximum and minimum temperatures in 1919 at a depth of 1 inch under the surface on burned and unburned plots.

#### SUMMARY

- (1) The data presented in this paper have been obtained in connection with pasture investigations conducted by the Kansas Agricultural Experiment Station. The experiments discussed were designed to study the effect of burning on the vegetation in pastures.
- (2) Areas were burned early each year for comparison with similar areas not burned. A number of small quadrats in each area were charted three times between the time of burning and July 1 each year. The results show that in the early part of the season there was considerably more growth of grasses on burned areas than on those unburned, thereby substantiating the popular opinion that burning causes growth to start earlier in the spring. In the second charting there was always more vegetation on the burned areas but the difference was not so great as in the first charting. The third charting was done when the greater part of the season's growth had been made. The differences found in the third charting were slight, showing that as the season advanced the vegetation on the unburned sections tended to catch up with that on the burned areas.



(3) Burning did not decrease the total number of grass plants, as is generally thought to be the case. The data show that at the end of four years there were 21 per cent more squares containing grass plants on the burned area than there were when the experiment started. On the other hand, the increase in the unburned plot was only 7 per cent. These data, while not final, indicate that the evil effects of burning, if any, are not always apparent in the first four years.

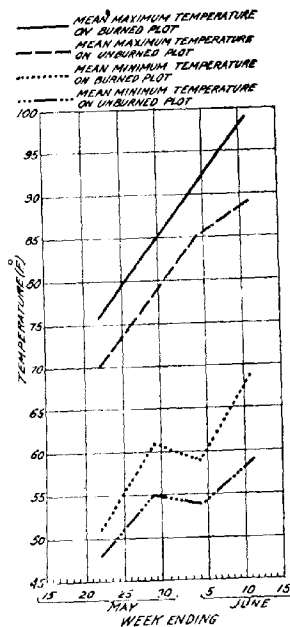


FIG. 1.—Graph showing weekly mean maximum and minimum temperatures in 1920 at a depth of 1 inch under the surface on burned and unburned plots.

while on the unburned one their number increased.

(7) Mean maximum soil temperatures at a depth of 1 inch averaged  $12.1^{\circ}$  F. higher on the burned plot. The mean minimum temperatures were also higher by  $4.1^{\circ}$  on the burned plot. Similarly, at a depth of 3 inches mean maximum and minimum temperatures were  $3.6^{\circ}$  and  $4.2^{\circ}$  higher, respectively, on the burned plot. These data may explain why growth starts earlier when the old vegetation has been removed by burning.

(8) The vegetation from each plot was cut about September 10 each year. The average yield was slightly greater on the unburned area. The difference, however, was slight, except during one season, and in another season it was in favor of the burned plot.

(9) The conclusion is that studies so far conducted have failed to show that burning is injurious. More extensive experiments with different types of vegetation and for a longer period of time must be conducted before final conclusions regarding the effects of burning can be arrived at.

(4) There was a decrease during the four years in the number of sedges on the burned area, while on the unburned plot a tendency to increase was observed.

(5) Burning caused a change in the composition of the grass type on the experimental area. Big bluestem (*Andropogon furcatus*) decreased on the burned plot, while on the unburned plot it showed a decided increase. On the other hand, burning increased the number of plants of little bluestem (*Andropogon scoparius*), although the number decreased on the unburned plot. Kentucky bluegrass increased much more rapidly on the unburned area. Bluegrass starts very early in the spring, and it appears that it may have been more easily injured than other grasses which start late. Maple plants were green when the burning was done. Side oats grama decreased on both burned and unburned plots.

(6) In 1918, when the experiments were started, both areas were practically free of weeds. In 1919 a great many were found on both burned and unburned plots. From this date on there was a decrease each year in the number of weeds on the burned plot.

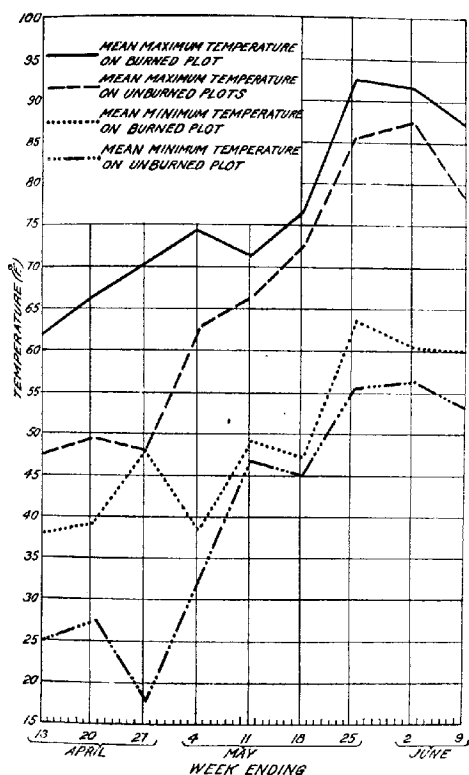
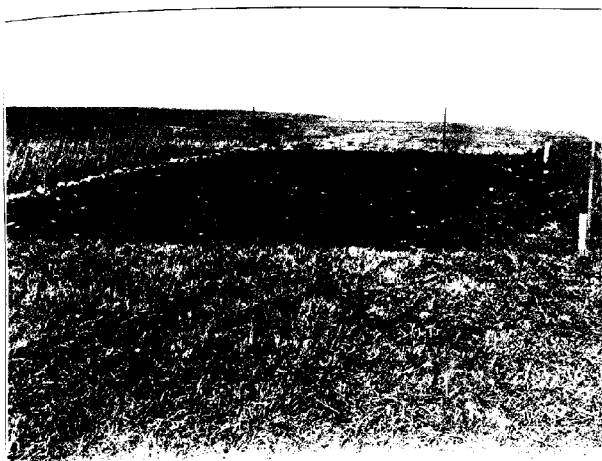


FIG. 4.—Graph showing weekly mean maximum and minimum temperatures in 1921 at a depth of 1 inch under the surface on burned and unburned plots.

PLATE I

A.—General view of the plot which was burned each year. The plot in the foreground is the one which was never burned. The illustration shows how close the latter area was cut to imitate grazing.

B.—Showing the vegetation on the unburned plot after cutting to imitate grazing. The portion on the right shows the original stand before the cutting was done.



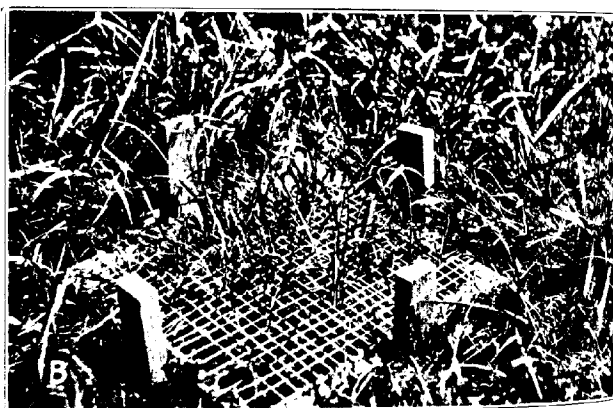
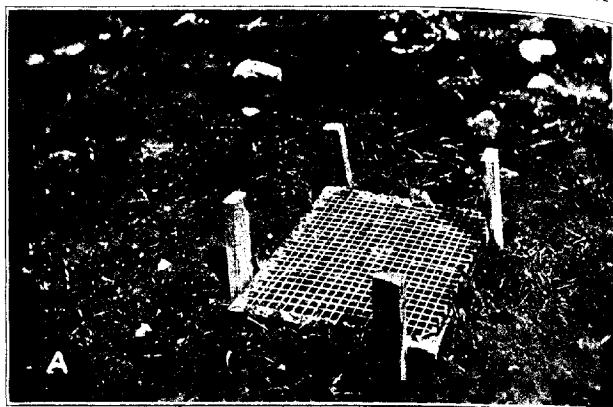


PLATE 2

A.—One of the 10 by 10 inch quadrats permanently staked and ready for the season's work. The interstices are one-half inch square. The area upon which this quadrat is located had just been burned when this photograph was taken.

B.—A view of the same quadrat as is shown in A, taken a few weeks after burning. Note the clean, vigorous growth and the absence of last year's litter.



# CONTROL OF LETTUCE DROP BY THE USE OF FORMALDEHYDE<sup>1</sup>

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## INTRODUCTION

The growing of head lettuce under glass has been developed to a very high degree in the Boston market garden district and other parts of Massachusetts. It is usually the main greenhouse crop, and not uncommonly the market gardener grows 4 to 6 acres under glass. In many of these houses the soil has not been changed for 20 years or more. As a result it has become so thoroughly infested with *Sclerotinia libertiana* Frickl., the fungus which causes drop, that the loss to the crop is sometimes enormous.

Investigations on this disease were begun by Stone and Smith (7, p. 3)<sup>3</sup> of the Massachusetts Agricultural Experiment Station as long ago as 1895. In the course of their work they devised a method of soil sterilization by the use of steam, and since 1900 this has been in more or less general use for the control of drop. The method has some serious disadvantages, however, which led in 1914 to the reopening of the investigation by the department of botany of the same station.

## REVIEW OF LITERATURE

There are few experimental data available dealing specifically with the control of *S. libertiana* by the use of formaldehyde. Smith (5) states that the mycelium of *S. libertiana*, growing on pieces of lemon crates, was killed by immersing it for 1, 5, and 10 minute periods in a 5 per cent formaldehyde solution. Treating the same fungus with formaldehyde as it occurs naturally in lettuce beds, Stevens (6) and Reddick (8, p. 200) report negative results, whereas Clinton's (4) results were of a positive nature. Since the completion of this work Beach (2) reports slight gains with the use of formaldehyde on out-of-doors lettuce beds. Also, *New Jersey Agriculture* (1) reports similar results with the use of formaldehyde on out-of-doors seed beds.

Although the literature contains conflicting reports on the efficacy of formaldehyde used as a soil disinfectant, our own preliminary tests indicated that the material might have considerable value in the control of lettuce drop. Consequently the work was concentrated on the use of formaldehyde in this connection, and extensive laboratory and greenhouse tests have been made. The work has included treatment of large commercial greenhouses on a practical basis.

<sup>1</sup> Accepted for publication Jan. 16, 1922. Published with the approval of the director, Massachusetts Agricultural Experiment Station.

<sup>2</sup> The writer is greatly indebted to Prof. A. Vincent Osmun, head of the Department of Botany of this station for his many helpful suggestions during the progress of this study.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 654.



## LABORATORY TESTS OF THE EFFECT OF FORMALDEHYDE ON SCLEROTIA AND MYCELIUM

## TREATMENT OF SCLEROTIA

Some very interesting data were obtained by treating sclerotia of *S. libertiana* in vitro for different periods of time with different concentrations of formaldehyde. Films of air covered portions of the surface of the sclerotia when first placed in the solution. This difficulty was overcome by vigorous agitation in distilled water for one-half hour immediately preceding treatment. Sclerotia specified as small were 1 mm. or less in diameter; those designated as large were over 1 mm. in diameter.

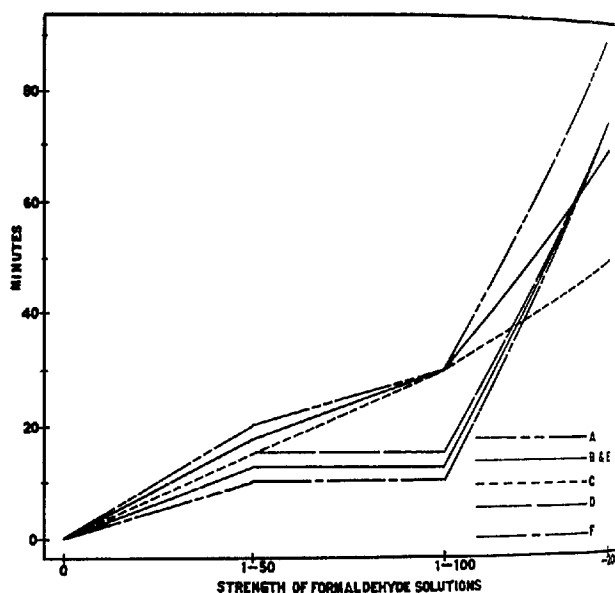


FIG. 1.—Graph made from Table I, showing the maximum and minimum time required in minutes to kill large and small sclerotia in 1-50, 1-100, and 1-200 formaldehyde solutions. Curve A shows the maximum time required to kill large sclerotia with these solutions; curve C shows the minimum time required to kill sclerotia; and curve B is an average of the total treatments made on large sclerotia. Curve D represents the maximum time required to kill small sclerotia with these solutions; curve E shows the minimum time required; and curve F is an average of the results obtained with small sclerotia.

Sclerotia (Tables I and III) were taken from diseased lettuce plants after they had "dropped" in the bed. The sclerotia were grouped into lots of nine each according to size. After shaking in distilled water they were transferred to 400-cc. air-tight glass jars containing formaldehyde solutions of the desired strengths,<sup>4</sup> allowed to remain for different periods after which they were removed under aseptic conditions and thoroughly washed three times in sterile distilled water. From this they were transferred to nutrient agar plates and the results of growth recorded (Tables I and III). Under these conditions, in a 1-50 solution the maximum time

<sup>4</sup> 1-50, 1-100, and 1-200 formaldehyde solutions were made by using one part of 37.3 per cent formaldehyde to 49, 99, and 199 parts, respectively, of distilled water.

required to kill small sclerotia was 15 minutes (Table I, treatments 1 and 3), while all large sclerotia were killed in 20 minutes (Table I, treatments 2 and 4). In a 1-100 solution the maximum time required to kill small sclerotia was 15 minutes (Table III; treatments 1, 3, and 8), whereas all large sclerotia were killed in 30 minutes (Table III, treatments 2, 4, 7, and 9). In a 1-200 solution the maximum time required to kill small sclerotia was 75 minutes (Table I, treatment 5), while all large sclerotia were killed in 90 minutes (Table I, treatments 6 and 7). In each concentration the small sclerotia were killed in less time than the large. Also, results show (fig. 1) that the longest time required to kill sclerotia of any size is inversely proportional to the strength of the solution. Furthermore, it should be noted (fig. 1) that a 1-100 formaldehyde solution is almost as effective as a 1-50 solution but that a 1-200 solution is much less effective than the stronger solutions.

Sclerotia of lots 1, 2, 3, 4, and 7 (Table I) were taken from plants that showed drop symptoms 10 days before the sclerotia were removed; lots 5 and 6 were taken 12 days after the first symptoms of drop were noticed on the plants.

TABLE I.—Effect of formaldehyde on sclerotia

Treatment.	Size of sclerotia, lots of 9.	Number viable in control, untreated.	Number viable after treatment.											
			3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	25 min.	30 min.	50 min.	75 min.	90 min.	120 min.
1. Formaldehyde, 1-50.	Small...	6	7	5	3	1	0	...	...	...	...	...	...	...
2. Formaldehyde, 1-50.	Large...	9	7	7	0	5	3	0	...	...	...	...	...	...
3. Formaldehyde, 1-50.	Small...	8	...	6	...	0	0	0	0	0	0	...	...	...
4. Formaldehyde, 1-50.	Large...	7	...	...	...	5	0	0	0	0	0	...	...	...
5. Formaldehyde, 1-200.	Small...	9	...	...	...	9	...	9	2	0	0	...	...	0
6. Formaldehyde, 1-200.	Large...	9	...	...	...	7	...	7	...	7	0	0	...	0
7. Formaldehyde, 1-200.	Large...	8	...	3	...	3	7	4	...	8	9	2	0	...

## TREATMENT OF MYCELIUM

The white aerial mycelium of pure cultures or of diseased lettuce plants was thoroughly moistened by transferring for a few minutes to sterile distilled water. It was then removed and treated with the desired strength of formaldehyde solution for different periods of time. At the end of the exposure the mycelium was removed aseptically, washed in sterile distilled water, and transferred to nutrient agar plates. The results are recorded in Table II.

Mycelium from pure culture was killed in 5 to 10 minutes in a 1-50 or a 1-100 formaldehyde solution, and in 15 to 20 minutes in a 1-200 solution. Mycelium from diseased lettuce plants was killed in 10 to 15 minutes in a 1-50 or a 1-100 formaldehyde solution (Table II). The mycelium of pure cultures was killed in less time than mycelium from diseased lettuce plants.

The curve of figure 2 shows that the 1-50 and the 1-100 formaldehyde solutions are equally efficient in killing the mycelium of *S. libertiana* and that the 1-200 solution is decidedly less effective than the other two. It is interesting to note that this held true in most cases throughout these experiments.

TABLE II.—Effect of formaldehyde on mycelium<sup>1</sup>

Treatment.	Number viable in control, untreated.	Number viable after treatment.						
		3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	30 min.
1. Formaldehyde, 1-50.....	9	9	8	1 & 7 1/2	2	0	0	0
2. Formaldehyde, 1-50.....	9	.....	1	.....	0	0	0	0
3. Formaldehyde, 1-50.....	9	.....	0	.....	0	0	0	0
4. Formaldehyde, 1-100.....	9	8	8	4	5	0	0	0
5. Formaldehyde, 1-100.....	9	.....	9	.....	0	0	0	0
6. Formaldehyde, 1-100.....	9	.....	4	.....	0	0	0	0
7. Formaldehyde, 1-200.....	9	.....	9	.....	9	4	0	0
8. Formaldehyde, 1-200.....	9	.....	9	.....	9	7	0	0

<sup>1</sup> The mycelium of treatments 1 and 4 was taken from diseased lettuce plants; all others from the surface of 8-day-old agar cultures. Nine lots of mycelium used in each case.

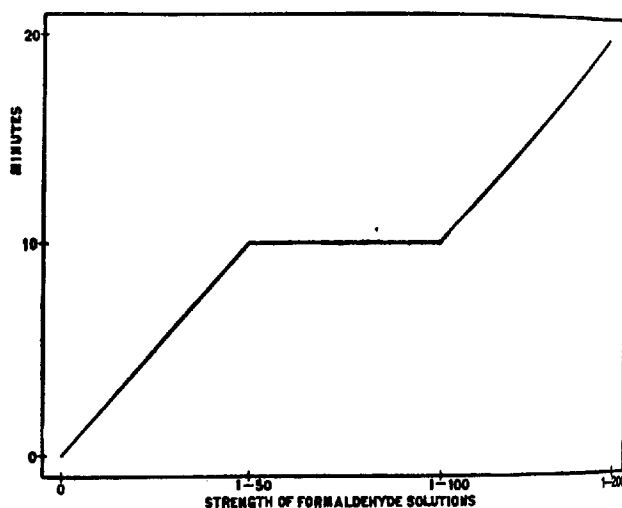


FIG. 2.—Graph showing the maximum time in minutes required to kill the mycelium of *S. libertiana* in 1-50, 1-100, and 1-200 formaldehyde solutions.

#### EFFECT OF ASPIRATING SCLEROTIA ON SUBSEQUENT TREATMENT WITH FORMALDEHYDE

To test the effect which surface and intercellular air might have on the action of formaldehyde on sclerotia, a number of sclerotia were aspirated in water in an aspirating bottle for  $\frac{1}{2}$  hour. Subsequently they were treated with a 1-100 formaldehyde solution, washed aseptically, and plated on nutrient agar. A comparison with the untreated controls shows that the process failed to shorten the time in which the sclerotia were killed. (Table III, treatments 6 and 7.)

Sclerotia of lot 2 (Table III) were taken 12 days after the first symptoms of drop were noticed on the plants; lots 1 and 3 were taken 14 and 18 days, respectively, after the first symptoms of drop were noticed on the plants. Lots 6 and 7 were aspirated for  $\frac{1}{2}$  hour previous to treatment, while lots 8 and 9 were kept moist in soil for 18 days previous to treatment. Lots 10 and 11 were air-dried for 18 days previous to treatment, and lots 4 and 5 for 16 and 35 days, respectively.

TABLE III.—Effect of formaldehyde on sclerotia

Treatment.	Size of sclerotia, lots of 9.	Number viable in control, untreated.	Number viable after treatment.											
			3 min.	5 min.	8 min.	10 min.	15 min.	20 min.	25 min.	30 min.	50 min.	75 min.	90 min.	100 min.
1. Formaldehyde, 1-100..	Small...	8	5	0	0	0	0	0	0	0	0	0	0	0
2. Formaldehyde, 1-100..	Large...	9	9	9	7	4	0	0	0	0	0	0	0	0
3. Formaldehyde, 1-100..	Small...	5	6	2	0	0	0	0	0	0	0	0	0	0
4. Formaldehyde, 1-100..	Large...	7	4	6	2	1	0	0	0	0	0	0	0	0
5. Formaldehyde, 1-100..	Large...	4	5	4	5	2	2	0	1	0	0	0	0	0
6. Formaldehyde, 1-100..	Small...	7	6	9	4	1	0	0	0	0	0	0	0	0
7. Formaldehyde, 1-100..	Large...	6	7	7	1	4	0	0	0	0	0	0	0	0
8. Formaldehyde, 1-100..	Small...	7	0	0	0	0	0	0	0	0	0	0	0	0
9. Formaldehyde, 1-100..	Large...	8	0	0	0	0	0	0	0	0	0	0	0	0
10. Formaldehyde, 1-100..	Small...	7	6	4	2	0	0	0	0	0	0	0	0	0
11. Formaldehyde, 1-100..	Large...	8	9	9	7	4	3	0	0	0	0	0	0	0

#### RELATION OF MOISTURE TO THE ACTION OF FORMALDEHYDE ON SCLEROTIA

Preliminary work showed that the time required to kill the sclerotia of this fungus in different concentrations of formaldehyde solution is correlated with desiccation. For further study of this phase one lot each of large and small sclerotia was kept in the laboratory in a dry bottle plugged with cotton, while similar lots were buried in moist loam soil. At the end of 18 days the four lots were removed and treated with 1-100 formaldehyde solution. Both small and large sclerotia kept under moist conditions described above were killed in less than 5 minutes (Table III, treatments 8 and 9). Small dry sclerotia were killed in 15 to 20 minutes (Table III, treatment 10), while large dry sclerotia were killed in 30 to 50 minutes (Table III, treatment 11). When the period of air-drying was extended to 35 days, large sclerotia were not killed by an exposure of 75 minutes (Table III, treatment 5). In all the experiments where sclerotia were stored under moist conditions the formaldehyde solutions were more effective, which, from the practical viewpoint, emphasizes the need of keeping the soil thoroughly moist from 5 to 10 days before treatment.

#### INHIBITORY EFFECT OF FORMALDEHYDE ON CULTURAL GROWTH OF *S. LIBERTIANA*

During the progress of this study it was found that the solutions of formaldehyde greatly inhibited subsequent growth on agar of sclerotia and mycelium which were not killed during the treatment—that is, growth was delayed by the treatment. It appeared that the action of

the formaldehyde on the mycelium was to produce partial dehydration of the cells from which there was slow recovery, or in the case of the sclerotia, the formaldehyde killed the outer, but not the inner cells, in which case the inner cells developed growth slowly because of their position. To illustrate the retarding effect of formaldehyde solutions on subsequent growth of sclerotia and mycelium a brief account is given of an average treatment with each strength.

**ONE-TO-FIFTY FORMALDEHYDE SOLUTION.**—Sclerotia were treated in the solution, washed aseptically, and plated on nutrient agar. Results taken 6 days later showed that sclerotia kept in the solution for 10 minutes made an average colony growth of 8 mm. in diameter, while the sclerotia kept in a solution of the same concentration longer than 10

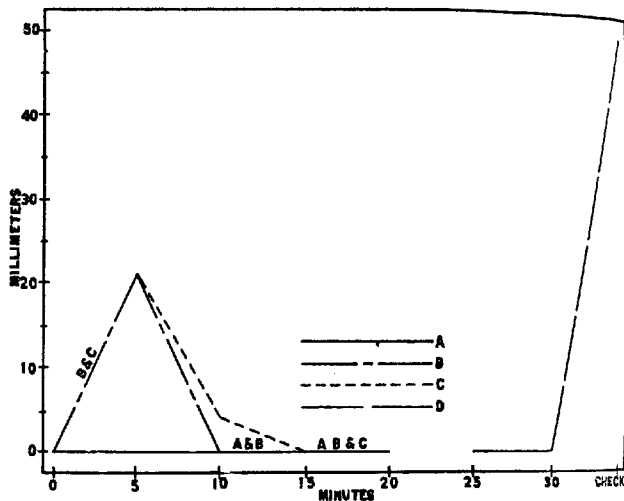


FIG. 3.—Graph showing the inhibitory effect of different concentrations of formaldehyde on the subsequent growth of mycelium treated and transferred to agar plates. Curve A is a straight line and shows that in a 1-50 solution growth was not evident after the shortest exposures; curve B shows similar results with a 1-100 solution; curve C with a 1-200 solution. There was no 25-minute exposure, hence, the skip in line A, B, C at this period. The growth of the colonies from the untreated mycelium forms the last part of each curve. All measurements taken at the end of the fourth day.

minutes were completely killed. Untreated sclerotia, cultured on the same kind of agar for 6 days, developed colonies averaging 32 mm. in diameter.

Ninety particles of mycelium from nutrient agar cultures were treated in the solution for different lengths of time, washed and plated on nutrient agar (Table II). Only 1 of the 90 particles showed growth. This one remained in the solution for 5 minutes and made a scant growth at the end of 11 days. Later the colony developed five sclerotia. The controls showed vigorous growth at the end of the first day, each colony producing from 2 to 3 dozen sclerotia.

**ONE-TO-ONE-HUNDRED FORMALDEHYDE SOLUTION.**—The method of procedure was the same as with the 1-50 solution. After 6 days, sclerotia kept in the solution for 10 minutes developed an average colony growth 26 mm. in diameter, while those exposed for 15 minutes produced an

average growth of 5 mm. Sclerotia exposed 20, 25, and 50 minutes showed no growth at the end of 6 days, but at the end of 21 days a few sclerotia treated for 20 and 25 minutes showed scant growth. The untreated sclerotia produced an average colony growth of 31 mm.

Nine out of 90 particles of mycelium showed growth (Table II); these remained in the solution only 5 minutes and showed no growth until the fourth day after they were transferred to the agar. The controls showed a vigorous growth at the end of the first day. Colonies from treated particles of mycelium developed sclerotia more slowly and fewer in number than those of the controls.

ONE-TO-TWO-HUNDRED FORMALDEHYDE SOLUTION.—The method of procedure was the same as with the 1-50 solution. Results taken 6 days after treatment showed that sclerotia kept in the solution for 20 minutes produced colonies averaging 17 mm. in diameter, while those exposed for 30 minutes developed colonies averaging 13 mm. Sclerotia exposed for greater periods were killed. Colony growth from the sclerotia of the untreated lot showed an average diameter of 43 mm.

Out of 90 bits of mycelium which were treated for different lengths of time, as shown in Table II, 18 were treated for 5 minutes. All of these developed with retarded growth. Eighteen were treated for 10 minutes, 14 of which were growing at the end of the fourth day and 18 at the end of the eleventh day. Of 18 treated for 15 minutes, none were growing at the end of four days, but 12 showed growth at the end of 11 days. Particles treated for 20 and 30 minutes were killed. The 18 controls showed vigorous growth at the end of the first day.

#### EFFECT OF FORMALDEHYDE ON SCLEROTIA AND MYCELIUM AT DIFFERENT DEPTHS IN SOIL

From the practical as well as the scientific viewpoint it is important to know to what depth sclerotia and mycelium are killed by a 1-100 formaldehyde solution applied to the surface of the soil. To determine this, sclerotia and mycelium were collected, placed in labeled copper wire baskets, and buried in a good lettuce soil at different depths (Table IV). Subsequently, the surface of the soil was treated with a 1-100 formaldehyde solution at the rate of 1 gallon to the square foot. At the end of 7 days the baskets were removed, opened, and 9 sclerotia from each depth washed in sterile distilled water and transferred to three nutrient agar plates. The particles of mycelium were treated in a similar manner. Growth was recorded at the end of the first, second, and third weeks. Final results from a large number of tests are recorded in Table IV.

Infested lettuce soils of commercial greenhouses contain sclerotia and mycelium of the causal fungus at varying depths. By the method just described it is possible to treat the organism under conditions practically the same as those in the soil of commercial houses and still recover the organism from the soil at will.

Table IV shows that with the exception of one particle buried at a depth of 16 inches all mycelium down to 20 inches was killed.

TABLE IV.—Effect of formaldehyde on sclerotia and mycelium buried at different depths in soil

Part of fungus treated.	Viable, sur- face.	Viable, 4 inches.	Viable, 8 inches.	Viable, 12 inches.	Viable, 16 inches.	Viable, 20 inches.	Moisture condition when buried.
Sclerotia from lettuce plants which showed first symp- toms of drop 8 days before sclerotia were treated.	o	o	o	o	o	o	Moist.
	o	o	o	o	o	o	
	o	o	o	o	o	o	
	o	o	o	o	o	o	
	o	o	o	o	o	o	
	o	o	o	o	o	o	
Sclerotia from infected lettuce plants.	o	o	1	5	3	3	Sclerotia air-dried for 15 days.
	o	o	2	o	4	o	
	o	o	o	1	o	2	
Sclerotia from pure culture 19 days.	o	o	o	2	o	2	Moist.
Sclerotia from pure culture 20 days.	o	o	o	2	8	5	
White mycelium from surface of infected lettuce plants.	o	o	o	o	1	o	
Mycelium in decomposing let- tuce leaves.	o	o	o	o	o	o	

As previously stated, the moisture content of sclerotia at the time of treatment is a very important factor in killing sclerotia with formaldehyde. This is again made evident in Table IV by comparing the effect of treatment on sclerotia in which a moisture content was maintained by contact with the host plant, and on air-dried sclerotia. In eight trials where sclerotia were removed directly from the host and treated, six showed all sclerotia killed down to 20 inches. In the other two trials the treatment was effective at a maximum depth of 12 inches. Sclerotia air-dried for 15 days were killed at a depth of 4 to 8 inches. Sclerotia taken from pure culture and treated in the same manner were apparently about as resistant to the action of formaldehyde solutions in the soil as sclerotia taken from lettuce plants. Obstructions in the soil such as pebbles, rocks, dry earth, etc., may act as shields preventing the formaldehyde from reaching the fungus, which possibly explains the variations in results.

A sclerotium has been defined as "a compact mass of hyphae in dormant state." The compactness of the dormant hyphae and the thick walls of the cells makes it extremely difficult for most disinfectants to penetrate to the interior cells of a sclerotium. Although formaldehyde is very penetrating it failed to kill the sclerotial stage of the fungus as quickly as the vegetative stage under the same conditions (Table IV).

#### TREATMENT OF GREENHOUSE SOILS FOR THE CONTROL OF *S. LIBERTIANA*

Since laboratory tests with formaldehyde proved so effective against the sclerotia and mycelium of *S. liberiana* it seemed advisable to test thoroughly its value for the control of this fungus as it occurs naturally in greenhouse lettuce soils. Therefore, extensive experiments have been

conducted along these lines, both in experimental and commercial greenhouses.

In eight successive lettuce crops grown in the experimental greenhouses at Amherst, the treated plots averaged 2 per cent drop, whereas the untreated plots averaged over 50 per cent drop (Pl. 1). Several large commercial houses were treated with results equally good.

In the course of the work it was shown that the treatment in all commercial houses should begin in the lettuce seed bed and that transplanting and permanent beds also must be treated. In all of the greenhouse experiments the soil was drenched with a 1-100 formaldehyde solution applied at the rate of 1 gallon to the square foot of soil surface. No covering was used over the soil after treatment, as it was found to be impractical on a large scale and the results showed that it added nothing to the efficiency of the treatment. If at any time sterilization is not complete or a house has only a few scattered cases of drop the location of the dropped plants can be marked with pot markers as the disease appears and the roots and tops removed. After the crop is harvested the spots where the diseased plants grew can be drenched with a 1-100 formaldehyde solution.

Lettuce plants 6 weeks old from seed may be set safely 8 days after the application of a 1-100 formaldehyde solution to the soil providing the soil is stirred 5 or 6 days after the treatment. But where only a few scattered spots are treated plants may be set as soon as the soil can be worked.

#### EFFECT OF FORMALDEHYDE ON SUBSEQUENT PLANT GROWTH

During the progress of this study it was manifest from a comparison of weights of lettuce plants of the treated and untreated plots that formaldehyde increased plant growth.

For example, 226 mature lettuce plants of a treated area in a commercial greenhouse weighed 164 pounds, while the same number of plants taken from an adjacent untreated plot weighed 132 pounds, which represents a gain of 23.6 per cent in favor of the plants grown on the treated soil. Clinton (4) also has noticed similar results on lettuce. Buddin (3) observed in his pot experiments with tomatoes that the crop was earlier and of greater bulk in the pots which had been treated with formaldehyde. That formaldehyde does increase plant growth must be accepted, but the exact reason for it has never been satisfactorily explained.

#### SUMMARY AND CONCLUSIONS

The maximum time required to kill small sclerotia with 1-50 formaldehyde solution in vitro was 15 minutes, and for large sclerotia 20 minutes. With 1-100 formaldehyde, small sclerotia, 15 minutes; large sclerotia, 30 minutes. With 1-200 formaldehyde, small sclerotia 75 minutes; large sclerotia, 90 minutes.

The maximum time required to kill both sizes of sclerotia is inversely proportional to the concentration of the formaldehyde solution.

In a 1-50 or a 1-100 formaldehyde solution in vitro, mycelium from pure cultures was killed in 5 to 10 minutes; in a 1-200 solution in 15 to 20 minutes.



Approximately the same length of time was required to kill sclerotia in both the 1-50 and the 1-100 solutions of formaldehyde; the same was true of mycelium.

Aspirating sclerotia for one-half hour in water and subsequent treatment in a formaldehyde solution in vitro failed to shorten the time in which they were killed.

Formaldehyde solutions are much more effective if the sclerotia are not in a desiccated condition. For this reason, the soil of commercial greenhouses should be maintained in a moist condition for 5 to 10 days immediately previous to the formaldehyde treatment.

The formaldehyde solutions greatly checked subsequent colony growth on nutrient agar of the sclerotia and the particles of mycelium which were treated in them.

In most cases the 1-100 formaldehyde solution killed sclerotia and mycelium 20 inches below the soil surface.

A 4-year test has demonstrated that a 1-100 formaldehyde solution is an effective soil fungicide when used to control *S. libertiana* in greenhouse soils. Sterilization is not always complete. In both experimental and commercial greenhouses the treated portions have not averaged over 2 to 4 per cent drop, whereas the unsterilized portions have averaged over 50 per cent drop.

In greenhouses that show only scattered cases of drop it is advisable to remove the diseased plants, mark their locations, and after harvest treat the infected area with a liberal application of a 1-100 formaldehyde solution. This will not delay work in the house, as the soil may be worked and planted the day following such treatment.

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# PLATE I

A.—A lettuce plot in the experimental house affected with drop in 1917. The ~~sax~~ year this plot was treated with a 1-100 formaldehyde solution. Nine crops of lettuce have since been grown in this bed without the loss of a plant.

B.—An untreated plot in the same bed as A and adjacent to it. Ninety-four ~~per~~ cent of the plants in this plot dropped.





## DISTRIBUTION OF PENTOSANS IN THE CORN PLANT AT VARIOUS STAGES OF GROWTH<sup>1</sup>

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### INTRODUCTORY DISCUSSION

Pentosans are known to be present in the cell walls of all green plants, in the bark and woody fiber of trees, in spices, fungi, mosses, various straws, tree and vegetable gums, fruits, and seeds. The wide occurrence of the pentosans in nature and the large percentage of furfural-yielding constituents in the cellular and skeletal structures of various plants has led many investigators to make a thorough study of these plant substances in the hope that some exact knowledge might be obtained concerning their origin and physiological function.

A study of the pentosan content of plants at various stages in their development discloses some significant facts. In most seeds the pentosans increase during the process of germination. This increase continues throughout the growth of the plant and is particularly marked in the fibrous portions such as the straw, the stem, or the leaf. The new tissue contains a smaller quantity of pentosans than the old; a young leaf may have only about two-thirds as large a quantity of pentosans as a dead and withered leaf. The formation of pentosans appears to parallel that of the cellulose and to be more particularly concerned with the structural requirements of the plant.

It is generally agreed that pentosans are not directly formed by assimilation of carbon dioxide. The increase in the actual quantity of pentosans in the germination stage indicates a conversion of hexoses into pentosans. This direct evidence for the conversion of hexosans into pentosans finds additional support in the discovery that pentosans are in almost every instance found with glucosans, while arabans are most often found together with galactans. (13, 14, 16, 6).<sup>2</sup> In many of the straws the xylose molecules are linked with the cellulose molecules such a firm complex that they can only be separated by a strong acid hydrolysis.

Arabinose-galactose complexes (galacto-arabans) have been found in gums, in arabinic acid, in cherry and peach gums, in gum tragacanth, and in coffee beans. De Chalmot (2) has pointed out the striking similarity between the stereoisomeric formulae of d-glucose and l-xylose, and between d-galactose and l-arabinose, and in view of this fact has proposed a theory to explain the conversion of hexosans into pentosans in the plant. According to this theory the pentoses are formed from the corresponding hexoses as a result of oxidation and decarboxylation of the terminal alcohol group. The proof for this theory has not been established, however, by experimental evidence.

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The question whether the pentosans may serve as reserve substances in the plants has been made a subject of study by numerous investigators. De Chalmot (3), Schöne and Tollens (12), and Götze and Pfeiffer (7) have concluded that the pentosans do not act as reserve substances. It has been shown that no decrease results in the pentosan content of plants during the different stages of development and hence the plant does not draw on the pentosans for the support of its life processes. Some exceptions appear to this general condition, as in the germination of the seeds of *Tropaeolum majus* (2), and in the decrease of pentosans in tree trunks (17) during the growing season. In a recent paper Tottingham, Roberts, and Lepkovsky (19) suggested that xylose-yielding substances may act as reserve carbohydrate in the metabolism of the apple tree. Miyake (9) determined the quantity of pentosans and methyl pentosans in the cotyledon and embryo of the germinated and ungerminated seeds of garden beans and soybeans and concluded that ordinarily the pentosans do not serve as reserve substances but that when the more easily utilizable material is used up the pentosans may function as reserve material. Schulze (13) and Ravenna and Cereser (11) are also of the opinion that in certain instances the pentosans may be used as reserve material.

Although the physiological function of the pentosans is still obscure and unsolved, it is doubtful whether their origin is the result merely of an accumulation of waste material. It is probable that they have some real importance in the formation of wood and in the formation of the skeletal structure of the various plants.

Associated with the pentosans are the methyl pentosans which have been found in mosses, algae, cereals, fungi, and in other plant material. Borghesani (1) made a study of the relationship between the amounts of pentosans and methyl pentosans in different species of soybeans and corn and found that this ratio was practically constant. However, little more than the wide occurrence and seemingly intimate relationship of the pentosans is known concerning the methyl pentosans.

A study of the pentosans and free pentoses in the corn plant at the various stages of growth seemed highly desirable, and these observations are described in this paper together with some data on the fermentation of the pentosans of green corn fodder by pure cultures of a number of bacteria.

#### EXPERIMENTS

Corn of the variety known as Golden Glow was planted in the greenhouse on January 15. The first plants were harvested on February 1 and were about 9 to 12 inches high. The plants were uprooted with the utmost care in order to obtain the entire root system and dry outer shell of the germinated kernel which is still attached to the plant at this stage of growth. These plants were not separated into tops and roots before determining the pentosan content. Some of the plants at the same time were harvested without the root system. Upon analysis it was found that the tops contained a considerably smaller percentage of pentosans than the whole plant. The plants of the succeeding stage were consequently divided into tops and roots.

The second series of plants was harvested on February 15. These plants were from 12 to 15 inches high and were just putting out their anchor or surface roots. They also still had the outer shell of the kernel attached to the root system.

The third series of plants was harvested on June 23 from a plot on one of the university farms. Because of the enormous increase in pentosans and dry matter in the plants which were harvested on March 9, it was felt that a series of plants should be selected which would represent a stage of growth intermediate between the series of February 15 and March 9, series 4. These plants were about 2 to 2½ feet high and were just putting out their anchor roots. On analysis, however, it was found that they were almost as far advanced as the series of March 9 and did not fit into the gap between the second and fourth series as well as could be desired.

On March 9, the fourth series of plants was harvested. These plants were about 3 feet high. All of the plants of the second, third and fourth series were divided into tops and roots.

The plants representing the next stage of growth were selected on March 26. They were about 5 to 6 feet high and were in the tassel stage. The plants were divided into roots, leaves, and stalks.

The sixth series of plants was harvested on April 12. These plants were in the silk stage and of about the same height as those of the preceding series.

On May 18 the plants of the seventh series were harvested. These plants were about 6 feet high and had produced ears which were in the milk stage.

The last series was selected on June 1. These plants were from 6.5 to 7.5 feet high, and the kernels were in the dent stage. The plants of the last three series were separated into roots, leaves, stalks, husks, cobs, and silk. In the last series the kernels were analysed separately from the cobs.

All the samples were thoroughly washed, dried, and weighed. A subsample of the material was ground to a fine powder and dried overnight at 105° C. in an electric oven. Duplicate samples of 1 gm. each were then taken, and a pentosan determination was made as described in the Kröber method (8). With these methods of sampling, grinding, and drying, duplicate determinations, checking to within 5 mgm. of chloroglucid were easily obtained. If a difference greater than 5 mgm. between duplicates was found, the determination was repeated. The results of this series of experiments are given in Table I.

TABLE I.—Pentosan content of the different parts of the corn plant at various stages of growth

[Calculated on the basis of 100 plants.]

Series No.	Age.	Stage of growth.	Part of plant.	Dry weight.	Pentosans.	Total pentosans.
	<i>Days.</i>			<i>Gm.</i>	<i>Per cent.</i>	<i>Gm.</i>
1	0		Kernels.....	29.6	7.4	2.2
	18	9 to 12 inches.....	Whole plant.....	23.7	15.4	3.6
2	31	12 to 15 inches.....	Tops.....	10.7	11.6	1.9
			Roots.....	11.2	19.5	2.2
3	40	2 to 2.5 feet.....	Tops.....	495.7	15.1	75.2
			Roots.....	74.3	18.2	13.5
4	53	3 feet.....	Tops.....	559.4	17.3	97.2
			Roots.....	81.4	17.3	14.1
5	70	Tassel.....	Leaves.....	1,106.6	19.9	220.7
			Stalks.....	1,016.6	19.1	194.2
			Roots.....	350.6	19.8	69.5



TABLE I.—Pentosan content of the different parts of the corn plant at various stages of growth—Continued

[Calculated on the basis of 100 plants.]

Series No.	Age.	Stage of growth.	Part of plant.	Dry weight.	Pentosans.	Total pentosans.
	Days.			Gm.	Per cent.	Gm.
6	87	Silk forming.....	Leaves.....	1,374.8	17.6	242.2
			Stalks.....	1,213.4	16.3	198.7
			Husks.....	751.2	15.3	114.9
			Cobs.....	488.6	9.0	44.1
			Silk.....	126.8	9.3	11.8
			Roots.....	338.6	20.3	68.9
7	123	Milk.....	Leaves.....	1,958.2	18.1	356.2
			Stalks.....	2,405.2	16.5	398.8
			Husks.....	1,128.2	25.6	288.8
			Cobs.....	4,201.0	17.4	732.6
			Silk.....	126.7	14.5	18.4
			Roots.....	458.0	19.6	90.0
8	137	Dent.....	Leaves.....	1,493.0	19.4	290.1
			Stalks.....	1,950.5	19.5	380.1
			Husks.....	1,019.0	33.0	336.1
			Corn.....	3,554.2	6.4	227.1
			Cobs.....	1,212.5	31.8	386.1
			Silk.....	93.0	79.9	18.1
			Roots.....	615.2	21.8	134.1

It is apparent that there is an immediate increase in the absolute quantity of pentosans in the plant. Even at the first stage when the shoots are only about 10 inches high the increase in the quantity of pentosans in the plant over the quantity in the kernels is 66 per cent. This indicates the conversion of starch or other dry matter into pentosans. At the second stage, when the shoots are only a few inches taller, the increase has mounted to 80 per cent. At the third stage the increase is several thousand per cent and thereafter increases enormously at each stage of growth. It will be noted that at the first stage the dry weight as compared with that of the kernels is about 20 per cent less, as is to be expected. At the next stage the dry weight has again reached that of the corn kernels and thereafter increases, except for the last stage where the dry weight is slightly less than in the preceding stage. It will be noted that throughout the growth of the plant the pentosans increase proportionately with the dry matter, the weight of pentosans being approximately one-sixth that of the dry matter.

Another fact of interest is the variation in the percentage of total pentosans in the different portions of the plant at the various stages of growth. From 7.4 per cent in the corn kernel the percentage has increased to 11.6 in the tops, and 19.5 in the roots of the plants at the second stage of growth. From this stage to maturity the roots show only a slight increase. The tops have not as yet reached the maximum percentage and increase rapidly as the plant develops. As the different parts of the plant appear they contain a low percentage of pentosans but there is a rapid increase as these parts develop. This is especially true of the cob, which shows an increase from 9 per cent to 32 per cent.

## METHYL PENTOSANS

The various parts of the corn plant were analyzed for methyl pentosans. The method of Ellett and Tollens (5) was used in which the precipitate of phloroglucid is extracted with 95 per cent alcohol at 60° C. until extraction is complete. Results identical with those reported in an earlier paper were obtained. In three determinations of total pentosans the decrease in the weight of the phloroglucid (about 0.208 gm.) varied from 0.0006 to 0.006 gm., the average being 0.0037 gm. These determinations were made on 1 gm. of dried material and therefore indicate the presence of only about 0.37 per cent of methyl pentosans in the tissue of the corn plant.

Because of the general distribution of methyl pentosans in plant tissue which has been reported by other investigations it was deemed desirable to check our procedure by a determination on rhamnose.

TABLE II.—Conversion of rhamnose into methyl phloroglucid under various conditions, and solubility of the phloroglucid

Sample.	Weight.	Yield of phloroglucid according to Kräfers tables.		Phloroglucid obtained.		Phloroglucid extracted by alcohol.
		Gm.	Gm.	Gm.	Per cent.	
Rhamnose.....	0.1	0.0587	0.0425	0.0425	72.4	0.0425
Arabinose.....	.2	.1364	.1041	.1041	76.3	.1041
Arabinose.....	.1	.0860	.0802	.0802	93.3	.0050
Rhamnose.....	.1	.0587				
Arabinose.....	.2	.1770				
Total.....		.2357	.2184	.2184	92.6	.0358
Plant material.....	1.0	.1672				
Rhamnose.....	.1	.0587				
Arabinose.....	.1	.0860				
Total.....		.3119	.2958	.2958	94.8	.0386
Plant material.....	1.0	.1672				
Rhamnose.....	.1	.0587				
Total.....		.2259	.2084	.2084	92.2	.0482

TABLE III.—Destruction of the pentosans of green corn stover by pure cultures of various organisms

Organism.	Source.	Pentose.			Pentosans destroyed	
		Gm.	Gm.	Per cent.		
Control.....		0.2146 .2150				
41-11.....	Soil.....	.1933 .2005	0.0215 .0143	10.0 6.6		
29.....	Sauerkraut.....	.2024 .2046	.0124 .0102	5.7 4.7		
124-2.....	do.....	.2030 .2095	.0118 .0053	5.4 2.4		
102.....	do.....	.2058 .2111	.0090 .0037	4.1 1.7		
31.....	Silage.....	.2101 .2075	.0047 .0073	2.1 3.3		
C-26.....	Green corn.....	.1878 .1904	.0270 .0244	12.5 11.5		
<i>Bacillus flavigena</i> .....	Fermenting cellulose.....	.1961 .1873	.0187 .0275	8.7 12.8		
3.....	do.....	.1904 .2159	.0244 .0111	11.5 5.1		
<i>Streptococcus lactis</i> .....	Milk.....	.2037 .1917	.0231 .0261	10.7 12.1		
<i>Bacillus coli communis</i> .....	do.....	.1887				

A series of experiments was carried out with varying amounts of rhamnose and arabinose alone and together with 1 gm. of plant material. The sugars were dissolved in distilled water, and aliquots representing the required amount were added to the plant material.

The yield of methyl phloroglucid which was obtained did not equal that calculated from Kröber's table. However, all of it was extractable with hot alcohol. A somewhat larger weight was extracted when the rhamnose was added to the corn tissue, indicating the small amount of methyl-pentosans in the plant material. The increase, 6 mgm., is approximately equal to the phloroglucid extracted in the regular determination. While these trials showed considerable variation, it may be assumed that no great amount of methyl-phloroglucid was formed and remained unextracted in the regular determinations. The results are given in Table II.

#### DESTRUCTION OF PENTOSANS IN PURE CULTURE

About 50 gm. of corn stover were ground to a fine powder. One gram of the air-dried material was weighed into small flasks, 50 cc. of yeast water were added, and about 2 gm. of calcium carbonate to alternate flasks. The flasks were then sterilized and inoculated. The results are given in Table III.

It is at once apparent that some of the organisms destroyed the pentosans to an appreciable extent. The maximum destruction was accomplished with the cellulose fermenter, *Bacillus flavigena*. Nearly the same percentage of pentosans was destroyed by *B. coli communis* and No. C-26, a chromogenic pentose fermenter isolated from green corn tissue.

TABLE IV.—Free pentoses in the corn plant at various stages of growth

Series No.	Stage of growth.	Part of plant.	Pentoses.	
			Gm.	Per cent.
4	3 feet.....	Tops.....	0.067	0.73
		Leaves.....	.062	.69
5	Tassel.....	Stalks.....	.065	.69
		Leaves.....	.047	.52
6	Silk forming.....	Stalks.....	.154	1.66
		Husks.....	.078	.87
		Leaves.....	.036	.39
		Stalks.....	.086	.92
7	Milk.....	Husks.....	.033	.35
		Cobs.....	.024	.25
		Leaves.....	.044	.47
		Stalks.....	.072	.76
8	Dent.....	Husks.....	.024	.25
		Corn.....	.017	.18
		Cobs.....	.025	.26

In an earlier paper (10) pentoses to the extent of 0.6 per cent were reported in the stalk and cob of every green corn which was analyzed according to the method of Davis and Sawyer (4). A decrease of 6 mgm. could therefore be accounted for by a destruction of the free pentoses of the corn stover. In addition 5 mgm. must be deducted for the difference in the duplicate weights of phloroglucid. A loss of 11 to 12 mgm., or 5 per cent, of pentosans must therefore be attributed to these two factors. A destruction of more than 5 per cent, on the other hand, may safely be attributed to the destruction of the pentosans themselves.

#### FREE PENTOSSES

Ten grams of the material were placed in an Erlenmeyer flask, together with approximately 5 gm. of calcium carbonate to neutralize the acidity and 100 to 150 cc. of 85 per cent alcohol. The samples were placed in a boiling water bath and refluxed. Two extractions of three to four hours were made. The insoluble material was filtered away and the alcohol evaporated. The residue was taken up with water, filtered, and finally distilled as usual with 12 per cent hydrochloric acid. The results are given in Table IV.

No extractions were made upon the plant material of the early stages because of the lack of material. It will be noted that the percentage of free pentoses is approximately the same in the leaves and stalks of series 4 and 6. The outstanding feature of Table IV is the high percentage of free pentoses in the stalks of the plants of the sixth series. Shaw and Wright (16) have published a table in which the amount of reducing sugar in the corn plant at the different stages of growth is given. At the stage at which the kernels are forming, which corresponds to series 6 in Table IV, they find the largest amount of reducing sugar, namely, 20.3 per cent. Coincidentally, the highest percentage of free pentoses was found in the stalks at this stage of growth.

Further evidence that free pentoses are contained in corn tissue was obtained by fermenting the alcohol-extracted sugars with yeast. The extracted sugars were made up with suitable nutrients and inoculated with a pure culture of yeast. Vigorous evolution of carbon dioxide ensued for a few days, but soon ceased. After 15 days the fermented liquid was analyzed for reducing sugars. From 500 gm. of corn tissue 3.54 gm. of sugar calculated as xylose remained unfermented by the yeast.

## SUMMARY

The pentosan content of the corn plant varies in different parts of the plant and at different stages of growth. From a percentage of 7.4 in the kernel, it increases throughout the stages of germination, growth, and reproduction until a percentage of 31.8 is reached in the cob at maturity. The several parts show minor fluctuations, but in general there is a steady increase both in the actual weight of pentosans and in the percentage of pentosans.

During the first 30 days after planting the weight of pentosans increased from 2.2 gm. to 4.1 gm. per 100 plants, although there was no increase of dry matter. Pentosans are evidently formed from starch or other dry matter. After 30 days there is a very rapid increase in both the pentosans and dry matter.

Only a trace (0.37 per cent) of methyl pentosans was found. Additions of rhamnose to the corn tissue were made, and the total pentosans were determined. Although a quantitative recovery was not obtained, the weight of phloroglucid soluble in hot alcohol was approximately equal to that obtained from both rhamnose and corn tissue separately.

Free pentoses were found to be present in the corn plant throughout its period of growth. They varied from 0.6 to 1.7 per cent of the dry matter. The maximum percentage was found in the stalks at the time when the kernels are forming. This is also the time at which the total sugars reach their maximum.

A destruction of the pentosans of green corn stover was obtained by pure cultures of a number of organisms. The maximum destruction was 12.8 per cent by a cellulose fermenter *Bacillus flavigena*. A chromogenic pentose fermenter occurring on green corn tissue also produced a considerable destruction of pentosans.

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# STIMULATING THE GROWTH OF AZOTOBACTER BY AERATION<sup>1</sup>

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## INTRODUCTION

Numerous theories and methods have been advanced for increasing the growth of the *Azotobacter* cell. Much attention has been directed toward the improvement of a medium, the dominating thought being that the common media used for azofication purposes are deficient in those elements which are necessary for the prompt development of the *Azotobacter* organism.

Söhngen (4)<sup>2</sup> states that in Beijerinck's medium only nitrogen and oxygen are lacking for the luxuriant growth of *Azotobacter*. A vigorous growth of these organisms could be induced by the addition of his (Söhngen's) colloids to this medium, the colloids causing a direct contact between the *Azotobacter*, oxygen, and nitrogen.

It is not doubted that the medium commonly employed in the cultivation of these organisms could be improved; there are few bacteriological media which could not be improved. On the other hand, it is not assumed that the medium exerts the greatest influence upon the tardy development of the *Azotobacter*.

The theory of Söhngen that the Beijerinck medium lacks only nitrogen and oxygen is here supported. Observations prompt one to believe that both these elements can be supplied by aerating the culture medium and that thereby a rapid and vigorous growth of *Azotobacter* can be promoted.

The influence of aeration upon nitrogen fixation by *Azotobacter* is not new. Its influence is taken advantage of in studying the azofication ability of the soil or pure cultures by inoculating media of shallow depth and large surface exposure. This Lipman (1) noticed in the cultivation of *Azotobacter vinelandi* Lipman, for the rate of nitrogen fixation increased with the increased surface exposed.

The relative importance of aeration upon the growth of *Azotobacter* can be surmised from some of its physiological activities. Its obligate aerobic characteristics as well as nitrogen requirements for the synthesis of cell protoplasm are significant. Inasmuch as nitrogen is lacking in the media, the utilization of atmospheric nitrogen is necessitated. The energy required for this assimilating process is supplied by the oxidation of a carbohydrate. The abundance of air therefore offers two highly essential elements, oxygen and nitrogen, both of which are required for the efficient metabolism of the cell.

Observations noted from the growth of *Azotobacter* cultures on dextrose-Ashby agar slants would not lead one to suspect the media as

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 677.



incapable of supporting rapid cell development. Such cultures have invariably shown a vigorous and heavy growth in 24 to 48 hours, a growth comparable to that exhibited by the common metatrophic forms growing on a nutrient medium. This rapid growth of *Azotobacter* on such agar slopes is attributed to maximum aeration.

The purpose of the present investigation was to develop a method for inducing a prompt growth of *Azotobacter* in liquid media. The importance of aeration in this respect is indicated by the following data.

#### METHOD OF AERATION

Hoffman (3) suggested the sand slope cultural method for supplying a maximum surface exposure for aeration. Allen (7) used the mechanical agitation method employed by Bonazzi (6) in his nitrification experiments and reported variable results with this method of aeration. He attributed the greatest influence to the chemical composition of the media.

The activity of organisms in many fermentation industries has been materially increased by aeration. Probably in most cases the interest has been focused upon the fermentation processes rather than on any direct interest in cell multiplication. However, within the last few years aeration has been employed commercially for the direct purpose of increasing the growth and thereby the yield of microorganisms. The cultivation of baker's yeast and the production of yeast protein in Germany by the fermentation of factory wastes is dependent upon this principle.

Aeration was accomplished in the following experiments by bubbling air vigorously and continually through the culture medium. The air was passed through the culture by attaching the culture flask equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the culture was reduced to a minimum by filtering the air through a sterile cotton filter and two or three sterile flasks of water. The air was thus filtered through cotton and rinsed in water before entering the culture flasks.

Several flasks of culture media could be easily aerated at the same time. Usually a cotton filter and wash bottle were placed between each two culture flasks. Little trouble occurred from contamination. A good grade of rubber tubing and tight-fitting connections are necessary, however. Flasks containing from 200 cc. to 250 cc. of media were used. The amount of air passed through the cultures was not measured, but a vigorous bubbling of the liquid was maintained continuously.

#### AZOTOBACTER CULTURES

Cultures of *Azotobacter* were isolated from samples of soil received from various parts of Kansas, Colorado, California, Iowa, and Mississippi. Flasks containing dextrose or mannite Ashby media were inoculated with the soil samples. Upon the formation of the characteristic surface growth Ashby agar plates were streaked. Dextrose-Ashby-agar slants were inoculated from well-isolated colonies, and repeated streakings of the cultures upon Ashby agar plates were made. A large number of the cultures were streaked consecutively from 1 to 12 generations. The utmost care was exercised in attempting to obtain and maintain pure cultures. Much difficulty was experienced in satisfying one's self of the absolute purity of a culture. This is accounted for by several justifiable reasons.

The purity of the culture is dependent practically entirely upon morphological considerations. The evident complex life cycle of *Azotobacter*, with its varied and fluctuating forms, makes a morphological differentiation indefinite, especially since our present knowledge pertaining to this life cycle, and its relative importance to the physiological activities of the cell is limited.

In all, 16 cultures of *Azotobacter* were used in these experiments. No attempt has been made to classify them as to species. In general, cultures 232, 6A, 12B, 9B, 8B, 1B, 10B, and 19 were all typically *Azotobacter* organisms producing a brown to black pigment. Culture 3A, 213, 2B, 5B, 11B, 9A, 222, and 3B were likewise typical, but pigment production by them was absent or doubtful. Culture 6A and 9B were isolated from Mississippi soil, 10B from California soil, 5B from Colorado soil, and 3A from Iowa soil. The remaining cultures were isolated from soil obtained from various parts of Kansas.

The inoculum used for seeding media was prepared by adding a portion of the emulsion from a young dextrose-Ashby-agar culture to a flask of dextrose-Ashby broth. This was aerated for two to four days. The amount of this starter usually used for inoculating the experimental medium was 0.05 per cent to 1 per cent of the medium seeded. In all cases a morphological analysis of the starter was made as a test for purity before using. The temperature for incubation was 30° C.

#### MEDIUM

The medium used in the following experiments, unless otherwise noted, was:

	Grams.
Tap water.....	1,000.0
Potassium phosphate ( $K_2HPO_4$ ).....	.5
Magnesium sulphate ( $MgSO_4$ ).....	.2
Sodium chlorid ( $NaCl$ ).....	.2
Dextrose.....	10.0

This solution was neutralized, filtered if necessary, and the required amounts placed in flasks and sterilized in the autoclav at 20 pounds pressure for 30 minutes. A pinch of calcium carbonate ( $CaCO_3$ ) was added to each flask before sterilization.

#### CHEMICAL ANALYSIS

Total nitrogen determinations and sugar analyses of the *Azotobacter* cultures were made at frequent intervals. In all cases the total nitrogen was determined by the usual standard methods. Unless otherwise noted, 50-cc. portions of the media were used in duplicate. The figures referred to in the tables denote the average of the duplicate analyses. Nitrogen is recorded in all cases unless stated as net gain in milligrams of nitrogen per 100 cc. of media. This, in other words, refers to the amount of nitrogen fixed per gram of dextrose, as 100 cc. of media contains this amount of sugar.

Sugar determinations were made according to the method proposed by Shaffer (5). The copper resulting from the reduced Fehlings was determined by colorimeter readings. Duplicate readings were made each time, and the average of these was recorded. As a general rule, 50 cc. of the media were used. The protein was precipitated and removed, the filtrate was diluted to 100 cc., and 20 cc. of this filtrate were used for reduction. The figures cited, unless so stated, refer to grams of dextrose per 100 cc. of medium.

## EFFECT OF AERATION UPON NITROGEN FIXATION

Flasks containing 250 cc. of media were inoculated with six *Azotobacter* cultures. Altogether, 36 flasks of media were used. Six flasks were inoculated with each respective culture. Three of the flasks for each organism were aerated and three were not. Examinations were made on the second, fourth, and sixth day.

The invigorating action of aeration is conclusively shown by these data, given in Table I.

An average of the figures from the six cultures give a net gain of 5.02 mgm. nitrogen per 100 cc. of the aerated media in two days as compared with 0.48 mgm. nitrogen in the nonaerated cultures. In four days the comparison indicates an average gain of 8.5 mgm. nitrogen in the aerated cultures to 1.24 mgm. nitrogen in the nonaerated cultures. The average net gain of the aerated flasks for six days was 10.35 mgm. nitrogen to 3.25 mgm. nitrogen for the nonaerated cultures.

TABLE I.—Effect of aeration upon nitrogen fixation

Culture.	Milligrams of nitrogen fixed.					
	Aerated cultures.			Nonaerated cultures.		
	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
1 BM.....	5.3	8.9	11.5	0	2.0	6.6
3 BM.....	4.9	14.2	15.9	0.34	1.2	6.0
222 M.....	6.0	8.0	11.0	.68	2.1	6.0
10 BP.....		7.1	8.1	.2	.2	.2
12 BE.....	4.3	5.6	8.4	.7	.7	.7
232 E.....	4.6	7.2	7.2	0	.....	.6
Average.....	5.02	8.5	10.35	.48	1.24	3.25

It is to be assumed that on account of insufficient surface exposure in the nonaerated cultures azofication would be tardy. Nevertheless the experiment demonstrates the stimulating effect of aeration and the possibility of cultivating the *Azotobacter* rapidly in large volumes of media.

To compare the relative efficiency of this method of aeration with shallow depth culture media the following experiment was performed. Eighteen 300-cc. flasks containing 50 cc. of dextrose media were inoculated with 0.5 per cent of inoculum of culture 19. These cultures were incubated and three flasks were removed for analysis each day for six days. Nitrogen determinations were made upon the contents of each of two flasks. The third flask was used for sugar analysis. Six 300-cc. flasks containing 250 cc. of the same media were inoculated with a similar proportion of culture and treated to forced aeration. One flask was removed each day for nitrogen and sugar determinations. This media contained no  $\text{CaCO}_3$ . The reaction of the medium was readjusted to a  $\text{P}_\text{H}$  of 7.0 to 7.4. The results are shown in Table II.

TABLE II.—Effect of aeration upon nitrogen fixation in culture 19

	1 day.			2 days.			3 days.		
	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.
Aerated.....	0.67	5.60	Alkaline	0.66	10.20	Alkaline	0	12.50	Alkaline
Nonaerated.....	.848	0	do.....	.60	4.50	do.....	Trace.	11.26	Do.

	4 days.			5 days.			6 days.		
	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.	Gm. dextrose.	Mgm. N.	Reaction.
Aerated.....	0	12.90	Alkaline	0	10.80	Alkaline	0	10.80	Alkaline
Nonaerated.....	0	12.06	do.....	0	10.80	do.....	0	10.80	Do.

The effect of the forced aeration is noticed in the first three days of incubation by both the nitrogen fixed and the dextrose fermented. The amount of nitrogen finally fixed in the shallow culture flask was practically as great as in the aerated culture. This would naturally be expected, however, as such cultures offered a maximum surface exposure. However, nitrogen fixation and dextrose consumption was more prompt in the aerated cultures.

The daily reaction of the cultures was alkaline, with possibly one exception which was questionable. This was the nonaerated culture on the fourth day of incubation. The  $P_H$  for the cultures reported alkaline averaged between 7.0 and 7.4.

#### EFFECT OF AERATION UPON DEXTROSE FERMENTATION

The ability of *Azotobacter* to fix nitrogen is dependent upon the energy derived from carbohydrate fermentation. The effect of aeration upon dextrose fermentation and the corresponding nitrogen fixation is demonstrated by the data in Table III.

TABLE III.—Effect of aeration upon dextrose fermentation and nitrogen fixation

Culture.	Aerated cultures.											
	1 day.		2 days.		3 days.		4 days.		5 days.		6 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
3H.....	0.54	1.3	0.26	4.3	0.07	6.1	0.04	5.6	0.04	.....	0.03	8.4
1H.....	.61	4.6	.09	4.6	.04	.....	.03	7.2	.03	.....	.03	7.2
Average.....	.57	2.9	.17	4.4	.05	6.1	.035	6.4	.035	.....	.03	7.8

TABLE III.—Effect of aeration upon dextrose fermentation and nitrogen fixation—Cont.

Culture.	Nonaerated cultures.											
	1 day.		2 days.		3 days.		4 days.		5 days.		6 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
12 BE.....	1	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7
232 H.....	1	0	0.8	0	0.8	0	0.8	0.7	0.7	0.6	0.7	0.7
Average.....	1	.35	.8	.35	.8	.7	.75	.7	.75	.65	.75	.7

Flasks containing 250 cc. of media were inoculated and incubated under aerating and nonaerating conditions. An aerated and nonaerated culture flask was analyzed each day for six days. The daily increase of nitrogen fixed as well as the daily consumption of dextrose in the aerated and nonaerated cultures is shown.

The marked effect of aeration upon *Azotobacter* is again indicated by these figures. In the aerated cultures the consumption of dextrose is very rapid. An average of 95 per cent of the original amount of dextrose disappeared within three days. Such is not the case in the nonaerated cultures. The average of the two cultures indicates that only 20 per cent of the dextrose was consumed within six days in the absence of aeration.

The comparative effect of aeration and nonaeration upon azotification and dextrose consumption is more clearly illustrated in figure 1 by the curves made from these data.

The striking effect of aeration is thus plainly evident, both in the nitrogen fixed and in the dextrose consumed. A close relationship is evident between the dextrose fermentation and azotification.

#### RATE OF NITROGEN FIXATION AND DEXTROSE FERMENTATION

Observation was made upon the comparative rate of nitrogen fixation and dextrose consumption in aerated cultures.

Allen (8) contributes data from one experiment and concludes that the results show that the rate of carbohydrate (dextrose) consumption by *Azotobacter* does not proceed in a manner similar to the rate of increase in cell numbers. Some of his cultures were agitated by mechanical means. This agitation appeared to hasten dextrose consumption as compared with those cultures which were not agitated.

The results obtained from six cultures of *Azotobacter* are shown in Table IV. The organisms were inoculated into a bottle holding 1,500 cc. of 1 per cent dextrose medium. The cultures were aerated two, four, six, and eight days. Total nitrogen and sugar determinations were made at the end of these periods by removing aseptically 150 cc. of the culture from the bottle. Curves plotted from the average of these results in figure 2, demonstrate a correlation between the rate of nitrogen fixation and dextrose fermentation. In general the results are similar to those indicated in figure 1.

The data representing the general average of the figures in the table are recapitulated in Table V. It will be noticed that the rate of azotification per gram of dextrose for each period of two days, is practically the same, namely, 6.90, 9.37, 8.34, and 9.74 gm. of nitrogen.

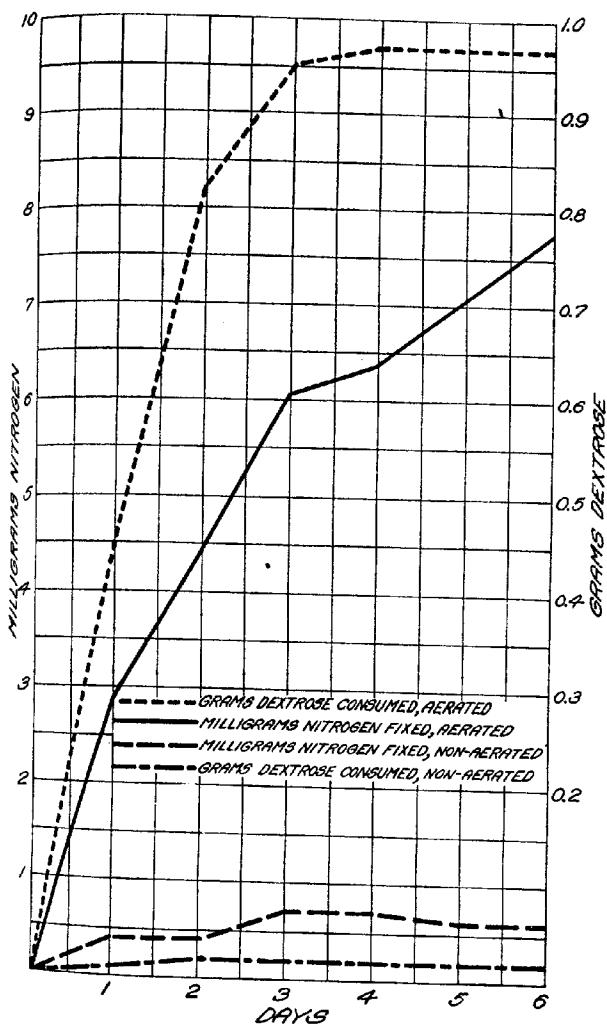


FIG. 1.—Effect of aeration on nitrogen fixation and dextrose fermentation.

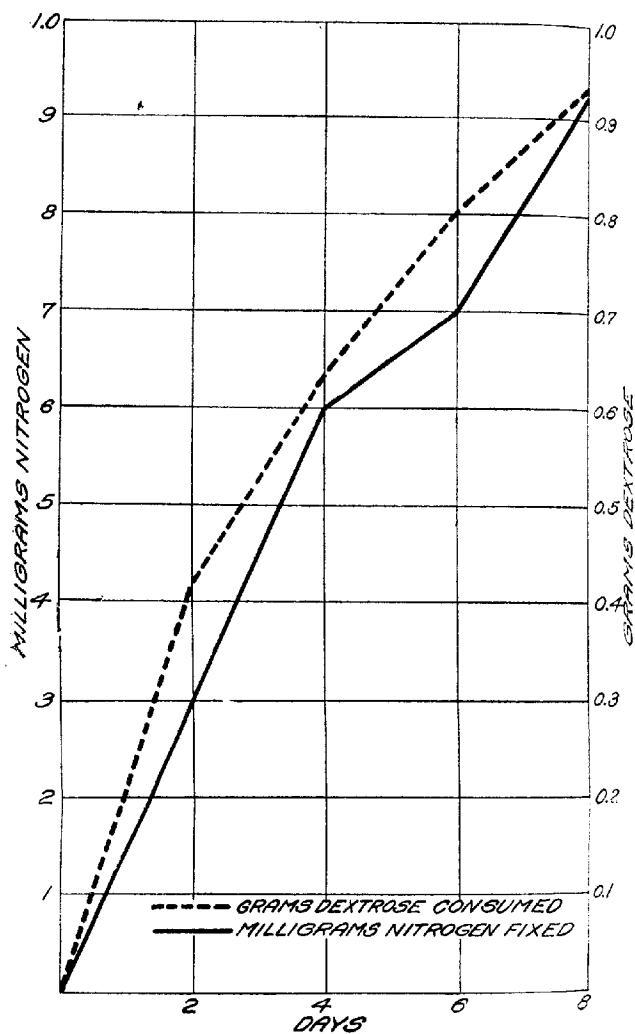


FIG. 2.—Rate of nitrogen fixed to dextrose consumed.

Examination also reveals that 42 per cent of the original amount of dextrose was consumed within two days, 22 per cent within four days, 17 per cent within six days, and 13 per cent within the eighth-day period, while 6 per cent was not fermented.

It also appears from these data that 31.6 per cent of the total nitrogen is fixed within two days, 33.8 per cent within the second period, 10.9 per cent within the third period, and 23.5 per cent within the fourth period. The amount of dextrose consumed in four days was 64 per cent of the sugar originally present, while the amount of nitrogen fixed within the same period was 65 per cent of the total nitrogen fixed. This periodical rate of nitrogen fixation and dextrose fermentation is noted in figure 3 by the curves plotted from these data.

Resummarizing the data in Table III as noted in Table VI shows that the rate of azofication per gram of dextrose per day is very uniform.

On the sixth day the amount of nitrogen recorded is greater than the amount actually fixed for this period as the nitrogen determinations were lost on the fifth day. The figures, therefore, in reality give the amount of nitrogen fixed between the fourth and sixth days.

During the first three days a fixation of 78 per cent of the total nitrogen occurred. The amount of dextrose fermented in the same period was 95 per cent of the original amount present.

The curves in figure 4, plotted from these data, exhibit curves similar to those noted in figure 3.

TABLE IV.—Rate of nitrogen fixation and dextrose fermentation

Culture.	2 days.		4 days.		6 days.		8 days.	
	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.	Gm. dextrose.	Mgm. N.
2 B.....	0.70	.....	0.49	6.6	0.28	6.7	0.10	7.0
1 B.....	.70	1.5	.49	8.6	.34	7.0	.13	7.4
3 A.....	.54	2.8	.24	8.0	.08	.....	0	11.6
5 B.....	.61	1.5	.49	5.6	.30	6.8	.2	9.8
11 B.....	.70	3.2	.49	4.9	6.21	5.3	0	10.0
3 B.....	.54	3.8	.49	4.9	.34	8.4	.3	.....
1 A.....	.50	2.9	.18	5.3	.04	7.7	.....	.....
13.....	.63	5.2	.32	5.6	.18	8.0	.....	.....
1 A.....	.38	5.3	.05	4.9	0	6.1	0	.....
Average.....	.58	2.9	.36	6.0	.19	7.0	.06	9.16

TABLE V.—Rearrangement of data from Table IV

	2 days.	4 days.	6 days.	8 days.
Total grams dextrose consumed.....	0.42	0.64	0.81	0.94
Total milligrams nitrogen fixed.....	2.90	6.00	7.00	9.10
Grams dextrose consumed each 2 days.....	.42	.22	.17	.13
Milligrams nitrogen fixed each 2 days.....	2.90	3.10	1.00	2.16
Rate of azofication per gram dextrose each 2 days.....	6.90	9.37	8.64	9.74



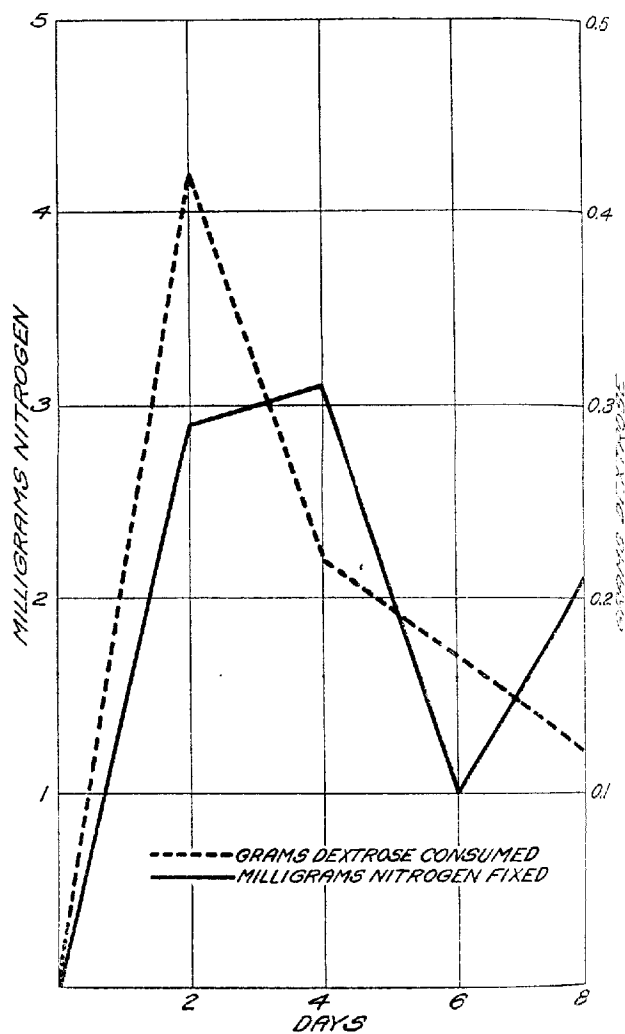


FIG. 3.—Daily amount of nitrogen fixed and dextrose consumed.

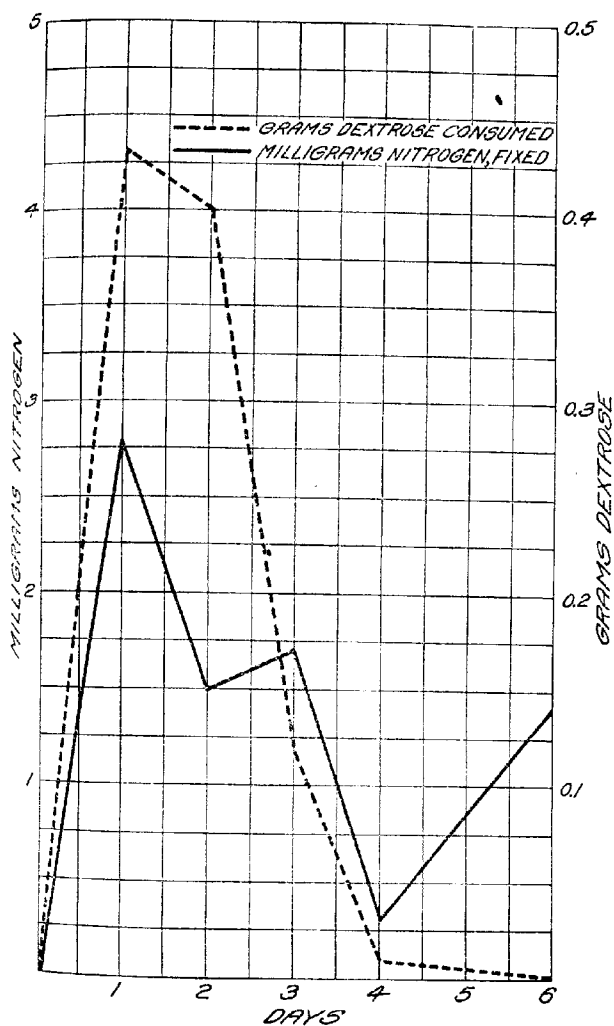


FIG. 4.—Daily amount of nitrogen fixed and dextrose consumed.

TABLE VI.—Rearrangement of data from Table III

	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.
Total grams dextrose consumed	0.43	0.83	0.95	0.965	0.965	0.97
Total milligrams nitrogen fixed	2.90	4.40	6.10	6.40	6.40	7.86
Grams dextrose consumed per day	.43	.40	.12	.015	0	.001
Milligrams nitrogen fixed per day	2.90	1.50	1.70	.30		1.40
Rate azofication per gram dextrose per day	6.51	5.30	6.42	6.56		8.94

EFFECT OF  $\text{CaCO}_3$ 

Calcium carbonate appears to be a universal constituent of the media used for the cultivation of *Azotobacter*. Lipman (2) asserts that calcium carbonate stimulates the growth of *Azotobacter* by either directly furnishing calcium or indirectly by making available more phosphorus, sulphur, and magnesium. Other investigators attribute its beneficial influence to the maintenance of a neutral reaction. Allen (7) in his studies on the composition of media for *Azotobacter* found  $\text{CaCO}_3$  to be essential.

For further investigation with the cultivation of *Azotobacter* a substance fulfilling all the requirements of a good medium was desired—namely, one that provides the proper food constituents, proper reaction, and is clear and free from sediment. The presence of  $\text{CaCO}_3$  interfered with the latter qualification. Preliminary experiments with a dextrose solution similar to the medium already mentioned, with and without  $\text{CaCO}_3$ , had furnished evidence supporting the doubtful value of  $\text{CaCO}_3$  for the growth of *Azotobacter* in pure culture.

Flasks containing 250 cc. of media with and without  $\text{CaCO}_3$  were inoculated with pure cultures of *Azotobacter* and aerated for six days. One flask of media was removed each day, and duplicate determinations of total nitrogen were made. The results are given in Table VII.

The averages of the analyses for two and four days indicate a slight increase in the amount of nitrogen fixed in the  $\text{CaCO}_3$  medium. However on the sixth day the non- $\text{CaCO}_3$  medium shows a slight increase over the  $\text{CaCO}_3$  solution. The slight increase of nitrogen fixed in one medium over the other is attributed to experimental error rather than to any marked effect of the media. These results are comparable with numerous others obtained in this laboratory. The conclusions are that with pure cultures undergoing a vigorous aeration the presence of  $\text{CaCO}_3$  in the media is not essential for the prompt growth of the *Azotobacter*.

It should be stated that the medium used in these experiments and in all other work where  $\text{CaCO}_3$  is not added was always corrected to a reaction near a  $\text{P}_H$  of 7.0 to 7.4. The reaction of such a medium inoculated with pure cultures of *Azotobacter* and aerated will vary but little.

TABLE VII.—Effect of  $\text{CaCO}_3$  upon nitrogen fixation

Culture.	Milligrams nitrogen per gram of dextrose.					
	2 days.		4 days.		6 days.	
	No $\text{CaCO}_3$ .	$\text{CaCO}_3$ .	No $\text{CaCO}_3$ .	$\text{CaCO}_3$ .	No $\text{CaCO}_3$ .	$\text{CaCO}_3$ .
BM.....	4.3	5.3	7.0	8.9	9.1	11.5
BM.....	4.3	4.9	9.4	14.2	14.9	15.9
122 M.....	4.9	6.0	6.1	8.0	15.9	11.0
10 BP.....	1.7	.9	5.2	7.1	9.1	8.1
12 BE.....		4.3	5.6	5.6	10.0	8.4
132 H.....	6.3	4.6	7.2	7.2	7.2	7.2
Average.....	4.3	5.0	6.75	8.5	11.03	10.35

## SUMMARY

- (1) A prompt and vigorous growth of *Azotobacter* can be induced in large quantities of liquid medium by sufficient aeration.
- (2) Aeration stimulates rapid nitrogen fixation by *Azotobacter*.
- (3) There exists a close correlation between the rate of dextrose fermentation and nitrogen fixation.
- (4) The presence of calcium carbonate is not essential in a medium used for aerating pure cultures of *Azotobacter*.

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